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Function of seed-bank ecology in mid-Atlantic semi-annual and perennial *Zostera marina* beds

Jessie C. Jarvis

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FUNCTION OF SEED-BANK ECOLOGY IN MID-ATLANTIC SEMI-ANNUAL AND
PERENNIAL *ZOSTERA MARINA* BEDS

A Dissertation

Presented To

The Faculty of the School of Marine Science

The College of William & Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

by

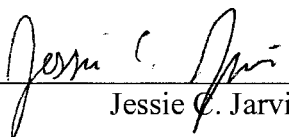
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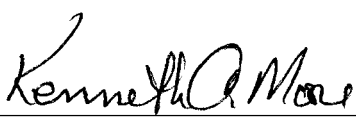
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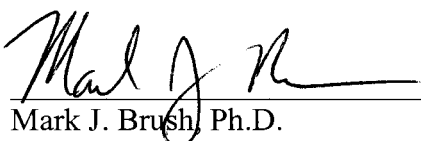


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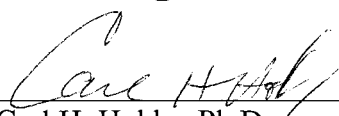
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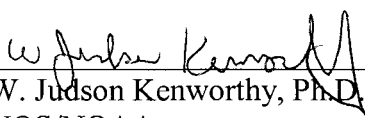
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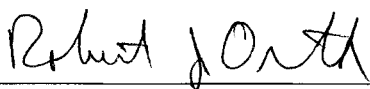
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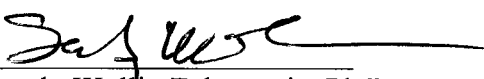
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ABSTRACT

The effects of water quality and sediment composition on mid-Atlantic semi-annual and perennial *Zostera marina* reproductive success, seed-bank viability, and seed germination were elucidated using laboratory and *in situ* experiments, quantitative field observations, and ecological model simulations. The sediment seed-bank was found to play a large role in the recovery of perennial *Z. marina* beds in the Chesapeake Bay and in the yearly re-establishment of beds in North Carolina which were determined to have a semi-annual life history. However, the resiliency provided by sediment seed-bank for both semi-annual and perennial *Z. marina* beds was limited as seeds remained viable for less than one year. When comparing the two life forms, semi-annual *Z. marina* beds produced a greater proportion of flowering shoots and more seeds than nearby perennial beds. Seed germination was significantly affected by sediment type and burial depth with maximum germination of seeds occurring in sediments containing $> 3\%$ organic content and buried at depths < 3 cm. Model simulations indicate that York River *Z. marina* beds are currently at their maximum temperature threshold and that projected increases of ≥ 1 °C in water temperature within the Chesapeake Bay may result in large scale declines. While the sediment seed-bank may provide a mechanism for recovery following one year of increased temperature stress, seed-banks are depleted following large scale germination events and may not provide resiliency to multiple consecutive years of stressful conditions. Further research into the interactive effects of sediment and water column conditions and seed physiology on seed viability are required to gain a more comprehensive understanding of seed-bank dynamics in *Z. marina* beds.

Monitoring of semi-annual and perennial *Z. marina* beds in North Carolina indicated that multiple life history strategies may be found within one *Z. marina* bed. Shoots within the semi-annual *Z. marina* bed germinated from seeds, a portion of seedlings flowered during their first year of growth, and all shoots completed their life cycle and died within one year of germination like a semi-annual plant; however, not all shoots flowered and shoots reproduced both sexually and asexually similar to a perennial plant. Since the individual plants found within the semi-annual bed did not display the all of the defining characteristics of either perennial or annual *Z. marina* life histories, this population cannot be completely described by either life history strategy. Research into the development of a semi-annual life history strategy for *Z. marina* within this site and the possibility of this form occurring at other geographic regions requires additional research.

Sexual reproduction is an important component of both semi-annual and perennial *Z. marina* populations that should be included in ecological studies and models. Although most perennial *Z. marina* beds rely on asexual reproduction as a primary form of bed maintenance, the ability to reproduce sexually is maintained and, as shown here, may play a large role in the recovery, maintenance, and expansion of these populations. For these reasons and due to the use of seeds in restoration of *Z. marina* beds within large systems such as the Chesapeake Bay, research into the dynamics of sexual reproduction within existing beds, a better understanding of seed physiology, and additional research into environmental effects (including the sediment) on seed germination and viability are essential.

INTRODUCTION

Background

Seagrasses, submerged marine angiosperms, are important components of global coastal ecosystems (Green and Short, 2003). Seagrass communities provide habitat, protection, and nursery functions for economically valuable fishery species (Duffy and Baltz, 1998; Richardson et al., 1998), serve as indicators of and modify local water quality conditions (Dennison et al., 1993; Moore, 2004), and decrease shoreline erosion by attenuating wave and current energy (Fonseca and Cahalan, 1992). Over the last 50 years seagrass populations have declined globally with little recovery (Orth et al., 2006).

Decline of seagrasses are attributed to a combination of natural phenomena (hurricanes, grazers, and diseases) and anthropogenic disturbances (Short and Wyllie Echeverria, 1996). The majority of global seagrass loss, estimated to be close to 33,000 ha, is due to both indirect and direct anthropogenic impacts (Green and Short, 2003; Ralph et al., 2006). These impacts include decreased light availability due to deteriorating water quality conditions (Dennison et al., 1993; Tamaki et al., 2002; Kemp et al., 2005) and increased sedimentation due to coastal development (Short and Wyllie Echeverria, 1996). Seagrass conservation and restoration efforts have increased in response to these global losses, however populations continue to decline (Orth et al., 2006). Limited seagrass restoration success combined with continually deteriorating water quality conditions requires an increased effort to understand how seagrasses become established, successfully survive, and reproduce in their present environments and under stressful conditions (Inglis, 2000).

Seagrasses maintain their populations primarily through clonal expansion and vegetative growth (den Hartog, 1970; Tomlinson, 1974; Hemminga and Duarte, 2000;

Rasheed, 2004). Clonal expansion sustains a successful genotype and removes the energetic cost of sexual reproduction (Ackerman, 2006). Despite the extra energy costs many seagrass species flower on an annual basis. Sexual reproduction increases genetic diversity (Harper, 1977), enhances recovery from large-scale declines (Whitfield et al., 2004; Waycott et al., 2005), and provides a mechanism for increasing the geographic range and establishment of new communities (Figuerola et al., 2002; Harwell and Orth, 2002; Källström et al., 2008). The role of sexual reproduction within a seagrass population depends on the balance between the ‘costs’, such as energy costs for producing flowering shoots, versus the ‘benefits’ like increased genetic diversity (Eckert, 2002). Due to the dominance of clonal expansion in seagrass beds the role of sexual reproduction in seagrass population dynamics is poorly understood.

Objectives

The main objectives of this research were (1) to quantify the effects of environmental conditions on seed germination, seed-bank viability, and the persistence of *Zostera marina* beds using both laboratory and *in situ* experiments; (2) to elucidate the role of sexual reproduction in the maintenance and recovery of established *Z. marina* beds; (3) to quantify and compare responses to various environmental conditions between semi-annual and perennial *Z. marina* populations; and (4) to develop an *Z. marina* production model to more accurately characterize how *Z. marina* beds respond to large scale disturbances and to determine what factors are most important for expanded research relative to reproduction. The overarching goal of this research is to add to the current understanding of the role of sexual reproduction in *Z. marina* bed maintenance

and recovery, and the potential role of seeds and seedlings in *Z. marina* conservation and restoration.

Zostera marina Description

A circum-global species in the Zosteraceae family, *Z. marina* has been found in both annual and perennial forms (Setchell, 1929; den Hartog, 1970) and is particularly suited for understanding the role of sexual reproduction in the decline and recovery of seagrasses. The wide distribution of *Z. marina* reflects an ability to live in a variety of environmental conditions. *Z. marina* shoots are found in habitats where water temperatures range from 0 °C to 35 °C (Biebel and McRoy, 1971), an average of 20 % of surface irradiance reaches the leaf surface (Duarte, 1991), and substrates range from sand to silty clay (Bradley and Stolt, 2006). Despite the ability to adapt to a variety of environments, many *Z. marina* populations are under stress from coastal development (Short and Wyllie-Echeverria, 1996) and have declined over the last several decades (Orth and Moore, 1983; Orth et al., 2006).

In response to disturbances, *Z. marina* populations increase sexual rather than asexual reproduction (Phillips et al., 1983a; van Lent and Vershuure, 1994). Sexual reproduction, or the production of reproductive shoots, varies with region and life history strategy resulting in total flowering shoot densities of 10 % to 100 % percent of total shoots (Silberhorn et al. 1983; Thayer et al., 1984; Meling-Lopez and Ibarra-Obando, 1999). *Z. marina* is monecious with male and female flowers produced on the same spadix found at the terminal end of the shoot (Setchell, 1929; Taylor, 1957; den Hartog, 1970). Reproductive shoot development is staggered such that inbreeding is minimized

(Ackerman, 2006). Production of reproductive shoots is variable within beds and across regions (Orth and Moore, 1986; Harwell and Rhode, 2007) and begins in March and April at lower latitudes with delayed production as latitude increases (Phillips et al., 1983b; Silberhorn et al., 1983). Phillips et al. (1983b) and Silberhorn et al. (1983) hypothesized that temporal variations in temperature and light are important environmental cues for flowering within *Z. marina* beds (Phillips et al., 1983b; Silberhorn et al., 1983). Successful pollination of flowering shoots can result in the production of 50 – 100,376 seeds m⁻² depending upon life history (Silberhorn et al., 1983; Meling-Lopez and Ibarra-Obando, 1999; Harwell and Rhode, 2007; Lee et al., 2007).

Although *Z. marina* beds rely primarily on asexual reproduction for maintenance of existing beds (Setchell, 1929; den Hartog, 1970; Short and Moore, 2006), seeds are important for dispersal (Churchill et al., 1985; Orth et al., 1994; Harwell and Orth, 2002; Källström et al., 2008), as a recovery mechanism for large scale declines (Plus et al., 2003; Greve et al., 2005; Lee et al., 2007), and most recently for use in *Z. marina* restoration efforts (Orth et al., 1994; Orth et al., 2000; Pickerell et al., 2005; Orth et al., 2006; Shafer and Bergstrom, 2008). Seed germination has been described as a potential limiting stage in sexual reproduction (Harper, 1977). Limitations to germination are attributed to the surrounding microenvironment which may lack the required signals to break seed dormancy and enhance germination (Baskin and Baskin, 1998; Woodin et al. 1998). For *Z. marina*, the primary environmental germination cues which perennial seeds have been most responsive to are changes in temperature (10-16 °C; Setchell, 1929; Taylor, 1957; Lamounette, 1977) dissolved oxygen (anoxic conditions; Churchill et al., 1992; Moore et al., 1993; Probert and Brenchly, 1999); and sediment organic content

(> 1 %; Short, 1987; van Katwijk et al., 1997; van Katwijk and Wijgergangs, 2004).

Seedling growth and establishment may also be a limiting stage in *Z. marina* sexual reproduction and requires further investigation.

Zostera marina Life Histories

Z. marina has developed perennial and annual life histories which allow the species to exploit habitats ranging from tide pools (Phillips et al., 1983a; Robertson and Mann, 1984; Keddy and Patriquin, 1978) and intertidal mud flats (Setchell, 1929; Harrison, 1993; van Katwijk and Wijgergangs, 2004) to subtidal zones in both temperate regions (Short and Moore, 2006; Lee et al., 2007) and in the tropical Gulf of California (Meling-Lopez and Ibarra-Obando, 1999; Santamaría-Gallegos et al., 2000). Although the species is found in a variety of forms, a majority of *Z. marina* populations are perennial and rely on asexual or clonal growth for bed maintenance and expansion (den Hartog, 1970). Understanding the environmental factors and population dynamics that affect the development of annual and perennial *Z. marina* beds may enhance our ability to manage existing *Z. marina* beds and increase the number of successful restoration attempts.

The current life history model for perennial *Z. marina* is driven by seasonal changes in temperature (Setchell, 1929; Phillips et al., 1983a; Thayer et al., 1984; Short and Moore, 2006). *Z. marina* remains in a state of quiescence when water temperatures are below 10 °C (Setchell, 1929). During this respiration and production are low (Nejrup and Pederson, 2008); however, *Z. marina* is not in an active stage of decay (Setchell, 1929). Vegetative growth is the dominant process when water temperatures range from

10 °C to 15 °C with flowering occurring once temperatures increase above 15 °C and continues as temperatures remain below 20 °C (Setchell, 1929; Phillips et al., 1983b; Silberhorn et al., 1983; Thayer et al., 1984). When water temperatures range from 20 °C to 25 °C *Z. marina* production is again reduced and the plants enter a period of heat rigor (Setchell, 1929; Nejrup and Pederson, 2008). Unlike cold rigor, heat rigor results in decreases in shoot density due to in part to decreased photosynthesis (Evans et al., 1986; Nejrup and Pederson, 2008). When water temperatures decrease below 20 °C perennial *Z. marina* shows a secondary increase in growth and production in surviving shoots before temperatures drop below 10 °C (Silberhorn et al., 1983; Thayer et al., 1984). The process begins again with vegetative growth once water temperatures increase above 10 °C the following spring (Setchell, 1929). Although the current perennial *Z. marina* life history model does include sexual reproduction (flowering), the model emphasizes clonal expansion and does not take the seed-bank, seed germination, or seedling growth and survival into account.

Annual populations of *Zostera marina* have been documented throughout the species range (Keddy and Patriquin, 1978; Phillips et al., 1983b; Robertson and Mann, 1984; Santamaría-Gallegos et al., 2000). Annual forms of *Z. marina* inhabit stressful environments such as tide pools and intertidal sediments where extreme temperature fluctuations and desiccation inhibit the growth and survival of perennial populations (Keddy and Patriquin, 1978; Harrison, 1979; Phillips et al., 1983a; Robertson and Mann, 1984; Keddy, 1987; Talbot, 2004). Shoots of annual *Z. marina* resemble typical perennial flowering shoots and annual *Z. marina* beds consist completely of flowering shoots with no production of vegetative shoots (Keddy and Patriquin, 1978; Keddy, 1987). All

annual shoots germinate from seeds and, unlike perennial *Z. marina* (Setchell, 1929; Taylor, 1957; Silberhorn et al., 1983), flower during the first year of growth (Phillips et al., 1983a; Robertson and Mann, 1984). Annual *Z. marina* populations have a compressed life cycle with seedlings germinating, flowering, producing seeds and dying in less than a year (Keddy and Patriquin, 1978; Phillips et al., 1983a; Santamaría-Gallegos et al., 2000). After seeds are produced, all above-ground and below-ground biomass is lost (Keddy and Patriquin, 1978; Phillips et al., 1983a; Harrison et al., 1993). Seeds remain within the sediment seed-bank until germination occurs the following year (Keddy and Patriquin, 1978; Phillips et al., 1983a).

In Chapter 1 two seagrass populations dominated by *Z. marina* in the Newport River and Back Sound, North Carolina were assessed monthly from July 2007 to October 2008 to (1) determine the dominant reproductive form (perennial/annual) of *Z. marina* at both sites and (2) to quantify differences in reproductive phenology (vegetative and reproductive shoot biomass, vegetative and reproductive shoot density, seed production, viable seed-bank density). Over a 15 month period I quantified differences in reproductive phenology between the semi-annual and perennial *Z. marina* populations and monitored changes in both water column (water temperature, salinity, dissolved oxygen, chlorophyll *a*, total suspended solids, dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) and sediment (organic content, % sand/silt/clay, redox, DIN and DIP) conditions between the two beds. By comparing measurements of semi-annual and perennial *Z. marina* bed growth I was able to document the presence of a semi-annual *Z. marina* bed in North Carolina and the presence of a form of *Z. marina* that cannot be completely characterized by either the annual or perennial life histories.

Zostera marina Sediment Seed-bank

Z. marina seeds do not typically germinate immediately after release (Moore et al., 1993) and can become incorporated into the sediment forming a seed-bank (Harwell and Orth, 2002). Sediment seed-banks are defined as a reservoir of seeds found within the sediment which are viable, or capable of germinating, and therefore are able to replace adult plants (Baker, 1989). In temperate areas such as Chesapeake Bay U.S.A. seeds are released in mid-May to June (Silberhorn et al., 1983) and germination may not occur until the end of October or November (Moore et al., 1993). Germination in the cooler fall season ensures a longer period of growth and increases successful seedling establishment before stressful summer water temperatures ($>25^{\circ}\text{C}$; Moore et al., 1997). Laboratory studies have shown that *Z. marina* seeds do not generally remain viable for more than 11 months although it is not clear how long seeds remain viable under *in situ* conditions (Harrison, 1991; Moore et al., 1993). How long *Z. marina* seeds remain viable in the sediment seed-bank, the effect of seed source (semi-annual or perennial) on seed viability and what factors (i.e. sediment organic content, sediment nutrients) affect the viability of seeds is unknown.

In Chapter 2 I quantified the effects of time, seed source, site, and sediment type on the viability of *Z. marina* seeds collected from both semi-annual and perennial *Z. marina* beds in two separate experiments within the Chesapeake Bay and the Newport River/Back Sound, North Carolina. In addition, I also quantified the effect of time and site on ambient seed-bank viability at all sites over a 15 month sampling period. Through the comparison of seed viability between semi-annual and perennial populations in an *in situ* experiment and between the experimental results and ambient observations, I was

able to quantify the effects of environmental conditions on *Z. marina* seed-bank viability. These results provide important information on the resiliency provided by seed-banks for both semi-annual and perennial *Z. marina* populations.

Zostera marina Seed Germination

Seeds of *Z. marina* vary in shape (oval to elliptical) and size (Wyllie-Echeverria et al., 2003). Although seeds are negatively buoyant, some may be dispersed over greater distances by gas bubbles (Churchill, 1985) or through rafting of reproductive shoots (Harwell and Orth, 2002; Källström et al., 2008). When released, seeds move only a few meters in the water column before they are deposited and incorporated into the bottom (Orth et al., 1994). Once deposited onto the sediment *Z. marina* seeds remain for several months and achieve maximum germination rates between water temperatures of 9 °C to 16 °C (Tayler, 1957; Moore et al., 1993) and under anoxic conditions (Moore et al., 1993; Probert and Benchly, 1999). Most germination studies have used perennial *Z. marina* seeds and it is not known if annual or semi-annual seeds germinate under similar conditions to perennial seeds.

In Chapter 3 I quantified maximum seed germination, time to germination, remaining seed viability, and seedling biomass between semi-annual and perennial *Z. marina* seed populations over a range of sediment types and depths. The comparison of both semi-annual and perennial seed germination over a variety of environmental conditions may provide important information on the adaptations of the differing reproductive forms. In addition, by quantifying viability of the remaining seeds, these

experimental results provide important information on the potential effects of the surrounding environment on *Z. marina* seed-bank viability.

Zostera marina Response to Disturbance

One role of sexual reproduction, through seeds and seed-banks, is to serve as a recovery mechanism after large scale population declines (Plus et al., 2003; Whitfield et al., 2004; Greve et al., 2005; Waycott et al., 2005). Global *Z. marina* declines have occurred over both short (weeks to months; Plus et al., 2003; Greve et al., 2005) and long (decades; Orth and Moore, 1983; Baden et al., 2003; Frederikson, 2004) time scales. Sudden large scale declines resulting in a wide-scale die back of *Z. marina* have been attributed to anoxic conditions (Greve et al., 2005) and water temperatures $> 30^{\circ}\text{C}$ (Moore and Jarvis, 2008). Initial recolonization in both studies was due primarily to seed germination and seedling establishment with minimal input by vegetative shoots. While seedlings were a large part of the initial return of *Z. marina* into these areas, it is not known if the seedlings will contribute to the continuation of the bed throughout the growing season and into the following year. Further understanding how *Z. marina* beds naturally recolonize will provide vital information for global conservation and restoration efforts (Kenworthy et al., 2006).

In Chapter 4 I quantified the re-development of three perennial *Z. marina* beds over two growing seasons following a large scale *Z. marina* decline in 2005. Perennial *Z. marina* beds in upriver and downriver regions of the York River were sampled monthly for changes in *Z. marina* abundance, shoot origin (seedling or surviving vegetative shoots), and seed-bank abundance and viability. By quantifying the recovery of *Z.*

marina populations, the viability of the sediment seed-bank, and the surrounding environmental conditions over time, the results of this chapter provide information on the importance of seedlings in initial bed recovery following a single disturbance and highlight the sensitivity of *Z. marina* beds in the Mid-Atlantic region to repeated stresses.

***Zostera marina* Sexual Reproduction Model and Synthesis**

In Chapter 5 I synthesized the data from the previous four chapters and highlighted areas of future research by developing a *Z. marina* production model with a sexual reproduction component. By combining the results of data from field measurements and experiments, perennial *Z. marina* seed germination, seed-bank viability, and seedling survival were modeled under conditions similar to those found immediately following the 2005 decline of Chesapeake Bay *Z. marina* in the York River, Virginia. The model quantified the role of sexual reproduction in perennial *Z. marina* bed recovery and projected the response of perennial *Z. marina* beds to episodic periods of stressful environmental conditions. In addition, the model was used to evaluate the resistance of *Z. marina* beds to, and recovery from episodic stresses such as increases in water temperature and decreased light availability (due to changes in suspended sediments and phytoplankton). Finally, by including data on sexual reproduction the model developed in this chapter may be able to more accurately predict the response of *Z. marina* beds to disturbance than existing models, focused only on vegetative biomass; thereby, allowing managers and policy makers to make more informed decisions relative to *Z. marina* habitat conservation and restoration.

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CHAPTER 1 : REPRODUCTIVE STRATEGIES FOR SEMI-ANNUAL AND
PERENNIAL ZOSTERA MARINA L. BEDS IN NORTH CAROLINA

Abstract

Two seagrass populations dominated by *Zostera marina* in the Newport River and Back Sound, North Carolina were assessed monthly from July 2007 through October 2008 to (1) determine the dominant reproductive form (perennial/annual) of *Z. marina* at both sites and (2) to quantify differences in reproductive phenology (vegetative and reproductive shoot biomass, vegetative and reproductive shoot density, reproductive shoot production, seed production, viable seed-bank density). The presence of an annual bed of *Z. marina* at Phillips Island (NC1) was confirmed by the complete loss of aboveground biomass at this site in November 2007 (0 g DW m⁻²) and again in September and October 2008 (0 g DW m⁻²). Although the *Z. marina* bed at NC1 was an annual bed not all shoots followed the annual life history. NC1 *Z. marina* shoots germinated from seeds, a portion of seedlings flowered during their first year of growth, and all shoots completed their life cycle and died within one year of germination like an annual plant; however, not all shoots flowered and shoots reproduced both sexually and asexually similar to a perennial plant. Since the individual plants found within the bed did not display all of the defining characteristics of either perennial or annual *Z. marina* life histories, the NC1 population cannot be completely described by either life history strategy and was characterized as a semi-annual population. Vegetative shoot density ($p < 0.001$), reproductive shoot production ($p = 0.002$), and viable seed-bank density ($p < 0.001$) were significantly greater at NC1 than a nearby perennial bed at NC2. Seed-bank viability was greater at NC1; however, seed-bank viability decreased over time with no viable seeds remaining after 6 months in the sediment. Water column temperature (°C), salinity (PSS), and dissolved oxygen (mg l⁻¹) followed seasonal patterns and did not

differ significantly between sites (temp $p = 0.711$; salinity $p = 0.527$; dissolved oxygen $p = 0.527$). The development of the semi-annual life history strategy at NC1 is not understood and further investigation into genetic variations and phenological response of this population to environmental conditions is required. These results highlight the need for annual replenishment of the seed-bank in both perennial and semi-annual life history forms of *Z. marina* and indicate that the resiliency provided by the sediment seed-bank may be limited.

Key Words: *Zostera marina*, semi-annual, seed-bank, phenology, North Carolina

Introduction

A member of the family Zosteraceae, *Zostera marina* is distributed circumglobally throughout the Northern Hemisphere (Setchell, 1920; Setchell, 1929; Green and Short, 2003; Short and Moore, 2006). A temperate species found in perennial and annual forms (Setchell, 1929; den Hartog, 1970; Phillips et al., 1983a), *Z. marina* has developed distinct life histories which allow the species to exploit habitats ranging from tide pools (Phillips et al., 1983a; Keddy and Patriquin, 1978) and intertidal mud flats (Setchell, 1929; Harrison, 1993; van Katwijk and Wijgergangs, 2004) to subtidal zones in both temperate regions (Short and Moore, 2006; Lee et al., 2007) and in the tropical Gulf of California (Meling-Lopez and Ibarra-Obando, 1999; Santamaría-Gallegos et al., 2000). Annual populations are not common as the majority of *Z. marina* populations are perennial and rely on asexual or clonal growth for bed maintenance and expansion (den Hartog, 1970).

Despite the prevalence of asexual reproduction, most *Z. marina* populations maintain the ability to flower (Setchell, 1929; den Hartog, 1970; Thayer et al., 1984). *Z. marina* is monoecious with male and female flowers produced on the same spadix at the terminal end of the shoot (Setchell, 1929; Taylor, 1957; den Hartog, 1970). Flowering shoots grow from the apex of the plant and flower development is staggered such that inbreeding is minimized (Ackerman, 2006). The proportion of flowering shoots and seeds produced within beds varies with habitat (Harrison, 1979; Phillips et al., 1983b; van Lent and Verschuure, 1995; Reusch, 2006; Harwell and Rhode, 2007) and life history (Phillips et al., 1983a; Mortie et al., 2007). Phenological cues such as temperature and

light are important environmental cues for flowering within *Z. marina* beds as flowering begins in April or May at lower latitudes with delayed production as latitude increases (Setchell, 1929; Philips et al., 1983b; Silberhorn et al., 1983).

The current life history model for perennial *Z. marina* is driven by seasonal changes in temperature (Setchell, 1929; Phillips et al., 1983a; Thayer et al., 1984; Short and Moore, 2006). *Z. marina* remains in a state of quiescence when water temperature is below 10 °C (Setchell, 1929) and respiration and production are low (Nejrup and Pederson, 2008); however, *Z. marina* is not in an active stage of senescence (Setchell, 1929). Vegetative growth is the dominant process when water temperature ranges from 10 °C to 15 °C with flowering occurring once temperatures increase above 15 °C and continues as temperatures remain below 20 °C (Setchell, 1929; Phillips et al., 1983b; Silberhorn et al., 1983; Thayer et al., 1984). When water temperature ranges from 20 °C to 25 °C *Z. marina* production is again reduced and the plants enter a period of heat rigor (Setchell, 1929; Nejrup and Pederson, 2008). Unlike cold rigor, heat rigor results in a decrease in shoot density due in part to decreased photosynthesis (Evans et al., 1986; Nejrup and Pederson, 2008). When water temperature decreases below 20 °C perennial *Z. marina* shows a secondary increase in growth and production in surviving shoots before temperatures drop below 10 °C (Silberhorn et al., 1983; Thayer et al., 1984). The process begins again with vegetative growth once water temperature increases above 10 °C the following spring (Setchell, 1929). Although the current perennial *Z. marina* life history model does include sexual reproduction (flowering), the model emphasizes clonal expansion and does not adequately account for the seed-bank, seed germination, or seedling growth and survival into account.

Annual *Z. marina* populations have been documented throughout the species range (Keddy and Patriquin, 1978; Phillips et al., 1983b; Robertson and Mann, 1984; Santamaría-Gallegos et al., 2000). Annual forms of *Z. marina* inhabit stressful environments such as tide pools and intertidal sediments where extreme temperature fluctuations and desiccation inhibit the growth and survival of perennial populations (Keddy and Patriquin, 1978; Harrison, 1979; Phillips et al., 1983a; Robertson and Mann, 1984; Keddy, 1987; Talbot, 2004). Shoots of annual *Z. marina* resemble typical perennial reproductive shoots and many annual beds consist completely of reproductive shoots with no production of vegetative shoots (Keddy and Patriquin, 1978; Keddy, 1987). All annual shoots germinate from seeds and, unlike perennial *Z. marina* populations (Setchell, 1929; Taylor, 1957; Silberhorn et al., 1983), flower during the first year of growth (Phillips et al., 1983a; Robertson and Mann, 1984). Annual *Z. marina* populations have a compressed life cycle with seedlings germinating, flowering, producing seeds and dying in less than a year (Keddy and Patriquin, 1978; Phillips et al., 1983a; Santamaría-Gallegos et al., 2000). After seeds are produced, all above-ground and below-ground biomass is lost (Keddy and Patriquin, 1978; Phillips et al., 1983a; Harrison et al., 1993). Seeds remain within the sediment seed-bank until germination occurs at the next optimum period (Keddy and Patriquin, 1978; Phillips et al., 1983a).

When comparing the production of reproductive shoots between *Z. marina* populations in Denmark, Olsen (1999) reported that annual beds produced a significantly greater proportion of flowering shoots and greater seed densities compared to perennial beds. Keddy (1987) described similar results were in Nova Scotia where annual beds produce up to seven times more seeds than perennial populations. As a spatial “bet-

hedging” strategy, annual plants produce greater densities of seeds to increase the chances of seeds finding suitable germination sites which also improve the odds of successful seedling establishment (Harper, 1977; Keddy, 1987; Symonides, 1988; Shipley et al., 1989; Harrison, 1993; Rees, 1996). However, by expending more energy on the production of seeds rather than on vegetative growth, annuals reduce the continuation of a successful genotype (Inglis, 2000). Perennial *Z. marina* expends less energy on sexual reproduction relying instead on the successful exploitation of a site by clonal growth (Inglis, 2000).

Once seeds are produced, they may be deposited within the bed (Orth et al., 2006); exported out of the bed on rafting flowering shoots (Harwell and Orth, 2002a; Källström et al., 2008), or lost to a variety of factors including general decay and predation (Fishman and Orth, 1996). A sediment seed-bank is defined as those seeds found within the sediment which are viable (capable of germinating) and therefore are able to replace adult plants (Baker, 1989). Seeds deposited in the sediments produce a transient (seeds remain for less than 1 year) seed-bank (Simpson, 1990; Harwell and Orth, 2002b; Jarvis, Chapter 3). Compared to the number of seeds produced in annual beds the contribution of yearly seed production to the sediment seed-bank is minimal and ranges from 5 % to 28 % (Mortia et al., 2007). Similar seed losses were reported in perennial *Z. marina* beds in Jindong Bay, on the Korean peninsula, where seed-bank densities varied inter-annually with highest densities occurring immediately after seed production (850 to 1780 seeds m⁻²) and lowest after the period of maximum germination (0 seeds m⁻²) (Lee et al., 2007). Overall 16 % of seeds produced were present in the seed-bank after a period of 12 months. The loss of viable seeds within annual and

perennial beds may actually be greater as these studies reported total seed density not viable seed density.

Environmental factors such as light availability (Dennison and Alberte, 1985; Dennison, 1987; Zimmerman et al., 1991), sediment and water column nutrient concentrations (van Lent and Verschuure, 1994), water temperature (Johnson et al., 2003; Moore and Jarvis, 2008) and sediment composition (Barko and Smart, 1986) have significant impacts on *Z. marina* growth, survival, and reproduction (van Lent and Verschuure, 1994). Although annual *Z. marina* is reported to dominate in areas with higher physical disturbance (Harlin et al., 1982; Robertson and Mann, 1984; van Lent and Vershuure, 1994) and in habitats with wide ranging environmental conditions (Keddy and Patriquin, 1978; Keddy, 1987; Talbot, 2004), the majority of phenological *Z. marina* studies have focused on perennial *Z. marina* due to the dominance of the life history throughout the majority of the species range (den Hartog, 1970; Short and Moore, 2006). Comparisons between the response of annual and perennial *Z. marina* populations to similar environmental conditions may provide a better understanding of the development of the two differing life-histories.

The Newport River and Back Sound regions of North Carolina are located in a transition zone between temperate and tropical seagrass regions at the southern limit of *Z. marina*'s range and at the northern limit of *Halodule wrightii* along the western Atlantic (Thayer et al. 1984, Short et al., 2007). At these two sites *Z. marina* dominates in the winter and early spring and the tropical *H. wrightii* dominates in the late summer and fall (Thayer et al., 1984; Short et al., 2007). Where the two dominant species overlap *H. wrightii* dominates the shallow intertidal zone while *Z. marina* is more prevalent in the

deeper subtidal regions (Thayer et al., 1984). In addition, the eurythermal and euryhaline seagrass species *Ruppia maritima* is also found to a lesser extent within seagrass beds in this region (Thayer et al., 1984). *Z. marina* growth and survival may be limited in the late summer when water temperatures can exceed 30 °C (Thayer et al., 1984).

Despite the presence of annual *Z. marina* beds at the southern limit of the species on the west coast of North America (Meling-Lopez and Ibarra-Obando, 1999; Santamaria-Gallegos et al., 2000), no annual *Z. marina* populations have been reported within North Carolina seagrass beds (Thayer et al., 1984). Recently one *Z. marina* bed at Phillips Island in the Newport River, in Carteret County, NC, was observed to die-back completely, re-establish with seedlings, and produce flowering shoot densities greater than the reported 28 % average for this region (Thayer et al., 1984; Kenworthy unpublished). Prior studies reported the *Z. marina* bed at Phillips Island was perennial, but recent observations suggest this site may have shifted from a perennial to an annual life history. It is the goal of this paper (1) to determine the dominant reproductive form (perennial/annual) of *Z. marina* at Phillips Island to confirm if it is an annual bed and (2) to compare differences in reproductive phenology (vegetative and reproductive shoot biomass, vegetative and reproductive shoot density, total produced seed density, total seed-bank density, and viable seed-bank density) between the annual and perennial beds.

I hypothesize that an annual form of *Zostera marina* has developed at Phillips Island, North Carolina because it is located near the southern limit of *Z. marina* distribution along the Western Atlantic. I also hypothesize that if the Phillips Island population is an annual form of *Z. marina*, then sexual reproduction (including reproductive shoot density, total produced seed density and viable seed-bank densities)

will be significantly greater and vegetative reproduction (vegetative shoot density) will be significantly lower at this site than perennial beds also found within this system. By evaluating phenological differences in reproduction between annual and perennial *Z. marina* populations within the same system, a more thorough understanding of the ecological consequences of in the responses of these two life histories to direct environmental conditions may be gained.

Methods

Site Selection

Two sites were selected in the Newport River and Back Sound, North Carolina for monthly monitoring based on historical *Z. marina* cover and observed dominant reproductive strategy (Thayer et al., 1984). Phillips Island (NC1) is located in the Newport River in Carteret County, North Carolina (NC1; 34° 43' N, 76° 41' W; Figure 1-1) and is a mix of *Z. marina* and *R. maritima*. Morgans Island (NC2), located approximately 14 km southeast of Phillips Island in Back Sound (NC2, 34° 66' N, 76° 52' W; Figure 1-1), is a mixed bed of *Z. marina*, *R. maritima*, and *H. wrightii*. Both sites are shallow, with water depth less than 2.0 m MLLW. In addition to water column characteristics *Z. marina* reproductive phenology was monitored at each site monthly from July 2007 through November 2008 while sediment characteristics were measured in July and December 2007 and in June and September 2008.

Water Column and Sediment Characterization

Bottom water temperature (°C) was monitored at each site every 15 minutes throughout the entire monitoring period with three HOBOWare Pro water temperature sensors. Salinity (PSS) and dissolved oxygen (mg l^{-1}) were measured during monthly site visits with a Yellow Spring Instruments, Inc. (YSI, Inc., Yellow Spring, Ohio) model 650 sonde. During each monthly site visit, three 500 mL water samples were collected by hand, filtered (Gelman Supor, $0.45 \mu\text{m}$), and frozen until analyzed for dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) with a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002). Water samples were also filtered and analyzed for chlorophyll *a* (Strickland and Parson, 1972) and total suspended solids (TSS). TSS was quantified from a well-mixed sample of known volume. The sample was filtered through a GF/F filter and the residue retained on the filter was dried to constant weight at $103 - 105^\circ\text{C}$. Final TSS values were reported as mg l^{-1} .

At each site, sediment samples were collected in August and December 2007 and in June and September 2008. These samples were analyzed for organic content, sediment exchangeable nutrients (DIN and DIP), percent sand/silt/clay, and redox potential. Five sediment cores (10.4 cm diameter by 10 cm depth) were collected at each site, divided into two 3 cm horizontal sections (0-3 cm, 3-6 cm), and each section was quartered. The first quarter was wet sieved ($63 \mu\text{m}$ sieve), washing slit and clay fractions into a graduated cylinder. After 24 hours, pipette analysis was performed with the filtrate to determine the clay (8 phi) and silt (4 phi) fractions of the sieved samples (modification of

Plumb, 1981). Dry weights of the aliquots were then compared and percent sand silt and clay fractions were determined.

Sediment percent organic matter was determined by drying one half of the sediment sub-section at 60 °C for 5 days or until a constant dry weight was reached. The samples were cooled in a desiccator and 10 g of sediment were then weighed and combusted at 500 °C for five hours. The sample was weighed again and percent organic matter calculated as the difference in weight before and after combustion (Erftemeijer and Koch, 2001). Sediment exchangeable nutrients of the remaining quarter of the sample were extracted with 2 M KCl, shaken for 1 hour, centrifuged 6 minutes at 4000 RPM, filtered (Gelman Supor, 0.45 µm), and frozen until analysis. Dissolved inorganic nitrogen (NH_4^+) and dissolved inorganic phosphate (PO_4^{-3}) were determined on thawed samples using a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002). In addition, daytime vertical redox (Eh) profiles to a depth of 10 cm on 3 cores for each sediment type were measured with a 21 cm platinum electrode. The probe was inserted into the top of the core and redox was measured at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 cm. Final readings were corrected for temperature relative to the reference electrode (Hinchey and Schaffner, 2005).

***Zostera marina* Characterization**

Five *Z. marina* biomass cores (22 cm diameter, 10 cm depth) were collected monthly from both NC1 and NC2 from August 2007 through October 2008. Samples were sieved in the field and all plant material was transported back to the lab on ice for

further analysis. Biomass samples were sorted as flowering shoots or vegetative shoots. Vegetative shoot density, reproductive shoot density, and the number of seeds per spathe were recorded. Vegetative shoots were then separated from the rhizome directly below the leaf sheath into above-ground and below-ground biomass. All biomass samples were dried at 60 °C until a constant dry weight to the nearest 0.01 g was reached.

***Zostera marina* Seed-bank Characterization**

The potential number of seeds produced at each site for all sampling periods was calculated as:

$$N_{ps} = S_s * I_m \quad (1)$$

Where N_{ps} = potential number of seeds produced, S_s = average number of seeds per spathe, and I_m = the average number of spathes m^{-2} (van Lent and Verschuure, 1994). At each site five additional sediment cores were collected quarterly and again in October and November 2008 to quantify total and viable seed-bank densities. All cores were wet-sieved (0.5 mm mesh); all seeds were collected, counted, and stored overnight in ambient seawater at 4 °C. Seed viability was tested using tetrazolium chloride which has increased accuracy and time efficiency over traditional germination tests (Lakon, 1949; AOSA 1981; Conacher et al., 1994; Sawma and Mohler, 2002). Seed embryos were removed from their seed coats and soaked in a 1 % tetrazolium chloride solution for 24 hours before examination on a dissecting scope at 10 x magnification (Conacher et al., 1994). Seeds with a pink to brown stained cotyledon and axial hypocotyl were considered viable (Taylor, 1957; Harrison, 1993). Mean monthly seed-bank viability was quantified as the percentage of total viable seeds collected. The percentage of seeds

retained within the sediment seed-bank was quantified compared to the potential number of seeds produced at each site:

$$\%R = \left(\frac{N_{ps} - N_{sb}}{N_{ps}} \right) * 100 \quad (2)$$

where % R = the percentage of viable seeds retained within the sediment seed-bank and N_{sb} = the total number of seeds collected from seed-bank samples.

Data Analysis

The effects of site and time on vegetative and flowering shoot biomass, the proportion of reproductive shoots, and the proportion of viable seeds in the seed-bank between annual and perennial life histories were analyzed with repeated measures Analysis of Variance (PROC GLM; SAS Institute Inc). Prior to analysis, data were transformed when necessary (biomass – square root transformation; proportion data – arcsine square root transformation), normality was confirmed, and homogeneity of variance was verified with Cochran’s test (Zar, 1999).

Differences in vegetative and reproductive shoot density, seed production, and the sediment seed-bank density between sites were analyzed using negative binomial regression with time and site as factors (PROC GENMOD: SAS Institute Inc.; Allison, 1999). For all significant ($p < 0.05$) model terms odds ratios were calculated using Wald chi square statistics (SAS; SAS Institute Inc). Likelihood ratio tests for all parameter estimates were also calculated and compared to the Wald Chi Square Statistics (Allison, 1999).

Differences in water column characteristics and sediment sand silt clay percentages between sites and over time were analyzed with nonparametric statistics as

the data were non-normally distributed (Kruskal-Wallis and Kolmogorov-Smirnov Tests; The SAS System for Windows, SAS Institute Inc.). All remaining sediment data were transformed when necessary and analyzed with repeated measures ANOVA to compare the effects of time, site and the interactions of these factors.

Results

Water Column and Sediment Characteristics

Water column temperature ($p = 0.711$), salinity ($p = 0.526$), and dissolved oxygen ($p = 0.527$) did not differ significantly between sites (Figure 1-2). Temperatures followed seasonal trends and were significantly different over time ($p < 0.001$). Daily mean temperature ranged from 7.2 ± 0.1 °C to 30.3 ± 0.1 °C at NC1 and from 4.8 ± 0.1 °C to 29.8 ± 0.1 °C at NC2. Dissolved oxygen patterns also followed seasonal trends with average values of 8.04 ± 0.58 mg l⁻¹ for NC1 and 8.81 ± 0.42 mg l⁻¹ for NC2. Salinities were nearly constant at both sites throughout the sampling period with no significant effect of time ($p = 0.115$). NC1 salinities averaged 32.4 ± 0.9 and NC2 salinities averaged 33.2 ± 1.4 throughout 2007 and 2008.

Water column chlorophyll *a* and TSS concentrations were significantly greater at NC1 than NC2 ($p < 0.001$) and were also significantly different over time (Chl *a* $p = 0.003$; TSS $p < 0.001$). Chlorophyll *a* mean concentrations were less than 6.0 µg l⁻¹ at both sites throughout 2007 and 2008. Water column NO_x (NO₂⁻ + NO₃⁻) and NH₄⁺ were not significantly different between sites ($p = 0.281$ and 0.999 respectively). DIP was significantly greater at NC1 ($p = 0.003$), however PO₄⁻³ concentrations were below 0.4 µg l⁻¹ for both sites. TSS followed a similar pattern to chlorophyll with concentrations

averaging less than 30.0 mg l^{-1} for both sites throughout the sampling period. Differences in chlorophyll *a* and TSS over time were driven by the August 2007 results where chlorophyll concentrations reached $10.72 \pm 1.39 \text{ } \mu\text{g l}^{-1}$ at NC1 and $15.61 \pm 4.25 \text{ } \mu\text{g l}^{-1}$ at NC2 and TSS reached $70.27 \pm 8.93 \text{ mg l}^{-1}$ at NC1 and $114.89 \pm 32.05 \text{ mg l}^{-1}$ at NC2.

Sediment organic content and sand/silt/clay percentages were the only sediment characteristics to differ significantly between sites. NC1 was the muddier site characterized by $21.6 \pm 1.1 \%$ silt and $7.3 \pm 0.5 \%$ clay fractions which was significantly greater ($p = 0.005$) than NC2 ($1.3 \pm 0.2 \%$ silt and $10.4 \pm 1.1 \%$ clay). In addition, NC1 also had significantly greater sediment organic content ($p < 0.001$) than NC2 (Table 1-1). Sediment DIN (NH_4^+ only, $p = 0.341$), DIP ($p = 0.063$) and redox ($p = 0.943$) did not differ significantly between sites. Daytime redox profiles indicate that, on average, the sediment retained some level of oxygen until depths of 2 cm where the levels dropped below 0 mV to depths of 10 cm.

***Zostera marina* biomass**

There was no significant difference in vegetative shoot above-ground biomass between NC1 and NC2 ($p = 0.477$; Table 1-2) although there was a difference over time ($p < 0.001$; Table 1-2). Above-ground biomass at both sites followed seasonal trends with peak biomass occurring in July in both 2007 and 2008 (Figure 1-3). Below-ground biomass was significantly greater at NC2 compared to NC1 ($p < 0.001$). NC1 above ground biomass reached $118.74 \pm 14.42 \text{ g DW m}^{-2}$ while the NC2 biomass peak was slightly lower at $97.25 \pm 22.53 \text{ g DW m}^{-2}$ (Figure 1-3). Seasonal biomass lows occurred in October and November in both 2007 and 2008. Above-ground biomass was

completely absent from NC1 in October 2007 and again in September 2008. While NC2 biomass decreased to the lowest recorded value, $13.80 \pm 5.00 \text{ g DW m}^{-2}$, in October 2007, there was biomass present during the entire sampling period at this site.

Reproductive shoot biomass was recorded at both sites from February to June 2008, reaching a maximum in April at both sites. NC1 reproductive shoot biomass ($30.93 \pm 2.41 \text{ g DW m}^{-2}$) was significantly greater than NC2 ($15.66 \pm 2.93 \text{ g DW m}^{-2}$; $p = 0.003$; Table 1-2, Figure 1-3). After reaching maximum values, reproductive shoot biomass quickly decreased at both sites before completely disappearing in July.

Shoot Density and Seed Abundance

Vegetative shoot density was significantly greater at NC2 than NC1 throughout the sampling in 2007 and 2008 ($p < 0.001$; Table 1-3). From July 2007 to November 2008 there were 2.5 times more vegetative shoots at NC2 than at NC1 (Figure 1-4). Vegetative shoot density followed seasonal patterns with peak shoot density occurring in early to mid-summer, depending on the site. Following the annual decline to zero in November 2007, NC1 peak vegetative shoot abundance occurred the following February with densities of $1,973 \pm 144 \text{ shoots m}^{-2}$. Immediately following the peak density period, NC1 vegetative shoot density decreased corresponding with the increase of reproductive shoots (February to June 2008) and continued to decline to zero by September 2008. Vegetative shoot density increased again with germination of new seedlings and reached $442 \pm 105 \text{ shoots m}^{-2}$ by November 2008. NC2 reached peak vegetative shoot density ($3,214 \pm 288 \text{ shoots m}^{-2}$) in April two months later than at NC1. While vegetative shoot

densities exhibited seasonal declines, shoot densities at NC2 were never below 531 ± 248 shoots m^{-2} (Figure 1-4).

Flowering shoot densities were similar between sites ($p = 0.889$; Table 1-3). NC1 flowering shoot density reached a maximum first in March 2008 with densities of 603 ± 157 flowering shoots m^{-2} (Figure 1-4). Densities of NC2 reproductive shoots peaked in May 2008 with a maximum of 463 ± 224 shoots m^{-2} . Despite the non-significant difference in reproductive shoot density between sites, the *Z. marina* bed at NC1 produced a significantly higher proportion of reproductive shoots during the 2007-2008 season ($p = 0.002$). For all months during the *Z. marina* flowering period, NC1 plants produced a greater proportion of reproductive shoots with up to 33 ± 3 % of total shoots flowering. During the period of maximum flowering (March to May 2008) only 26 ± 13 % of all shoots in the *Z. marina* bed at NC2 were flowering (Figure 1-4).

Due to the non-significant difference in reproductive shoot density, the potential number of total seeds produced also did not differ significantly between sites ($p = 0.092$; Table 1-3). While the proportion of reproductive shoots at NC1 was significantly greater than NC2 the overall total number of reproductive shoots, the number of rhipidia per shoot, and the average number of seeds per spathe were similar resulting with a non-significant difference between sites in the number of potential seeds produced (Table 1-4). While seed production was not significantly different, on average NC1 produced 17,000 more seeds per m^{-2} or 1.7 times more seeds than NC2.

Seed-bank

The greater abundance of seeds produced at NC1 than NC2 resulted in a 2.2 times greater density of seeds in NC1 compared to NC2 seed-banks ($p < 0.001$; Table 1-3). Compared to the observed total seed production in each site in 2008, less than 1 % of seeds produced were retained within the sediment seed-bank at NC2 and only 2 % were retained in NC1. Seed-bank densities were greatest in the samples collected in the fall of 2008 for NC2 and immediately after the end of the flowering shoot season in NC1 (Table 1-5). Seeds were always present in the seed-bank at both sites, although NC1 seed-banks were significantly depleted following the large germination event in November 2008. Of the less than 2 % of seeds retained within the sediment seed-bank at both sites, seed viability ranged from 0 to 13 ± 13 % for NC2 and from 0 to 33 ± 21 % for NC1. On average, densities of viable seeds were 27.2 times greater at NC1 than NC2 ($p = 0.003$; Table 1-3). At NC1 seed viability was greatest in the period just prior to the fall germination event (Table 1-5).

Discussion

The results presented here quantify the presence of a semi-annual *Zostera marina* population at Phillips Island in the Newport River, NC. While the bed was annual and regenerated from seeds on a yearly basis, the individual plants found within the bed did not display all of the defining characteristics of either perennial (Setchell, 1929) or annual (Keddy and Patriquin, 1978; Phillips et al., 1983a) *Z. marina* populations; therefore, the NC1 population cannot solely be described by either life history strategy. All *Z. marina* shoots were germinated from seeds, a portion of seedlings flowered during their first year

of growth, and all shoots completed their life cycle and died within one year of germination like an annual plant; however, not all shoots flowered and shoots reproduced both sexually and asexually, similar to a perennial plant. Therefore, the form of *Z. marina* documented here is characterized by a semi-annual history strategy which employs aspects of both perennial and annual forms of *Z. marina*.

Phillips et al. (1983a) observed *Z. marina* beds along the eastern Pacific which also incorporated aspects of both annual and perennial reproduction. Pacific *Z. marina* populations found in intertidal habitats characterized by increased environmental extremes and a greater frequency in physical disturbances produced significantly greater flowering shoots compared to perennial populations located in subtidal habitats. Phillips et al. (1983a) concluded that an increase in sexual reproduction indicated a shift from a perennial competitive life history to a perennial-annual life history although the plants were observed throughout the year. Furthermore they concluded that the increase of flowering in these perennial plants was related to environmental stress (Phillips et al., 1983a). Like the populations described in Phillips et al., (1983a) the proportion of flowering shoots at the semi-annual NC1 bed was significantly greater than NC2. However, there was no significant difference in measured water column characteristics between NC1 and NC2 in 2007 and 2008. Therefore, the presence of a semi-annual form of *Z. marina* at NC1 could not be directly related to measured environmental conditions.

Similar to the results presented here for populations located at the southern limit of *Z. marina* distribution along the western Atlantic, multiple life-histories have been observed at the southern limit of *Z. marina* distribution along the eastern Pacific (Phillips et al., 1983a; Meling-Lopez and Ibarra-Obando, 1999; Santamaría-Gallegos et al., 2000).

The presence of the semi-annual life history in *Z. marina* beds in the Gulf of California has been related to water temperatures greater than 30 °C which inhibit the growth and survival of perennial *Z. marina* (Meling-Lopez and Ibarra-Obando, 1999; Santamaria-Gallegos et al., 2000). Although late summer water temperatures across both NC sites reached maximum daily water temperatures of 30.3 ± 0.1 °C at NC1 and 29.8 ± 0.1 °C at NC2, almost 10 °C greater than the optimum range of 10 to 20 °C for perennial *Z. marina* growth and survival (Nejrup and Pederson, 2008), perennial *Z. marina* was observed at NC2 during all sampling dates in 2007 and 2008. In addition, maximum above-ground biomass 97.25 ± 22.53 g DW m² for NC2 was comparable to other perennial *Z. marina* beds in North Carolina (106 g DW m² to 200 g DW m²; Penhale, 1977; Kenworthy et al., 1981; Thayer et al., 1984). Therefore, unlike the *Z. marina* populations located at the southern limit of the species distribution along the eastern Pacific, temperatures do not seem to completely inhibit the growth and survival of perennial *Z. marina* in North Carolina.

In 2007 and 2008, the perennial bed at NC2 produced had 2.7 times more non-flowering shoots than the semi-annual site. Maximum vegetative shoot density of $3,214 \pm 288$ shoots m⁻² at the perennial site are similar to reported values in the Chesapeake Bay, Virginia ($2,918 \pm 970$ shoots m⁻²; Orth and Moore, 1986) indicating that vegetative shoot densities in the mixed life history bed at NC1 were lower than mid-Atlantic perennial beds in general. The reduction in vegetative shoot density may be related to the greater production of flowering shoots at NC1 compared to regional perennial *Z. marina* beds. NC1 *Z. marina* beds produced several hundred more flowering shoots m⁻² (603 ± 157 shoots m⁻²) than NC2 (463 ± 224 shoots m⁻²) or Chesapeake Bay (424 ± 170 shoots

m⁻²) *Z. marina* beds (Orth and Moore, 1986). As a result, potential seed abundances were up to 17,000 seeds m⁻² greater at the mixed life history site than regional perennial beds. Greater seed abundances within the semi-annual *Z. marina* bed at NC1 increased the ability of the bed to re-establish on a yearly basis from seed. Perennial beds do not re-establish on an annual basis; therefore, the *Z. marina* bed at NC2 may have reduced sexual reproduction and increased vegetative reproduction to increase the chances of survival of a successful clonal genotype (Inglis, 2000).

Z. marina at NC1 produced double the reproductive shoot biomass (30.93 ± 2.41 g DW m⁻²) compared to the perennial site at NC2 (15.66 ± 2.93 g DW m⁻²). In addition, within 11 months shoots within the semi-annual; life-history *Z. marina* population at NC1 were able to germinate, flower, and produce seeds similar to annual *Z. marina* beds (Keddy and Patriquin, 1978; Phillips et al., 1983a; Santamaria-Gallegos et al., 2000). This is substantially faster than perennial shoots within the same region which require up to 18 months before producing reproductive shoots (Setchell, 1929; Silberhorn et al., 1983; Thayer et al., 1984). The production of reproductive shoots during the first year of growth provides an annual supply of seeds to the bed and surrounding areas. Seeds within the bed will increase the density of the sediment seed-bank, providing a mechanism for re-establishment (Leck et al., 1989; Orth et al., 2000; Jarvis Chapter 3), while seeds transported out of the bed by rafting of reproductive shoots may disperse *Z. marina* into new areas increasing the distribution within the region (Harwell and Orth, 2002a; Källström et al., 2008).

Unlike annual populations, the seedlings at NC1 reproduced both sexually and asexually. As a result, the maximum proportion of reproductive shoots (33 ± 3 %) was

lower than the 100 % expected of annual populations (Keddy and Patriquin, 1978; Robertson and Mann, 1984; Santamaria-Gallegos et al., 2000). Despite the lower flowering shoot abundances, *Z. marina* at NC1 produced greater seed densities than NC2 resulting in the average production of $53,513 \pm 7,365$ seeds m^{-2} . As with flowering shoot densities, NC1 maximum potential seed abundance was much lower than the 78,224 seeds m^{-2} reported for annual beds in Nova Scotia (Keddy, 1987) and the $100,376 \pm 18,765$ seeds m^{-2} reported for annual beds in the Sea of Cortez, Mexico (Meling-Lopez and Ibarra-Obando, 1999). Based on the results presented here; the cost of reproducing both asexually and sexually in the semi-annual life-history bed at NC1 may have resulted in a reduction in the number of seeds produced compared to annual beds. However, by reproducing both sexually and asexually, NC1 beds were able to increase the sediment seed-bank therefore increasing the resiliency of the bed to disturbance (Leck et al., 1989; Jarvis, Chapter 4) while also maximizing successful genotypes within the bed (Inglis, 2000). Therefore, *Z. marina* populations at NC1 may be able to exploit a variety of conditions.

Fall sediment seed-bank density at NC1 represented less than 2 % of potential seeds produced in 2008. Seed-bank densities are crucial during this time of year as this is the maximum period of germination for *Z. marina* seeds in the mid-Atlantic region (Silberhorn et al., 1983; Thayer et al., 1984). Similar losses of seeds have been reported for both annual and perennial *Z. marina* beds throughout the species distribution (Santamaria-Gallegos et al., 1999; Harwell and Orth, 2002b; Morita et al., 2007). Within the Gulf of California 25 % of seeds produced by annual beds were estimated to be lost while still attached to the reproductive shoot (Santamaria-Gallegos et al., 1999). In Ago

Bay, Japan 78 % of seeds were estimated to be lost from the bed with remaining seed-bank densities of intact seeds ranging from 219 ± 103 seeds m^{-2} to $1,157 \pm 360$ seeds m^{-2} (Morita et al., 2007). These losses may be the result of dispersal (Källström et al., 2008), decay (Morita et al., 2007), predation (Fishman and Orth, 1996), or germination (Harper, 1977; Jarvis, Chapter 2).

When seed viability is taken into account, the loss of seeds was even greater at the semi-annual site as the total percentage of viable seeds within the seed-bank decreased from 33 ± 21 % immediately following the 2007 seed release to 0 % after the maximum fall germination period in the same year. While the viability of the sediment seed-bank was greater at NC1 compared NC2 in both 2007 and 2008, viability of all seeds decreased over time at both sites with no viable seeds collected within 6 months of seed production. The greater viability of the sediment seed-bank at NC1 with the semi-annual life history strategy may be a reflection of a bet-hedging strategy where the production of more seeds may result in a greater potential for germination and bed re-establishment (Keddy, 1987; Symonides, 1988; Shipley et al., 1989; Harrison, 1993; Rees, 1996).

Regardless of life history, seeds must remain viable in the sediment seed-bank until conditions are favorable for germination and newly germinated seedlings can replace adults within the established population (Baker, 1989; Murdoch and Ellis, 2000). Laboratory experiments have shown that when kept in a liquid medium, perennial *Z. marina* seeds remain viable for less than one year (McMillan, 1983; Harrison, 1991; Moore et al., 1993). The *in situ* observations of both perennial and semi-annual *Z. marina* beds presented here also indicated that seeds remain viable for less than one year when incorporated into the sediment. The production of a transient seed-bank by semi-

annual life history strategy *Z. marina* beds has large implications for the long-term survival of these populations. Flowering within *Z. marina* beds is influenced by a variety of factors including temperature (Setchell, 1929) and photoperiod (Phillips et al., 1983a). Reductions in flowering in response to variable environmental conditions may result in lower seed densities within the bed, lower viable seed densities within the sediment seed-bank and decrease the overall chances for successful germination. As semi-annual beds rely solely on seed germination for bed re-establishment, reductions in the sediment seed-bank could have extreme negative consequences.

Conclusions

The results reported here support a modification of the life-history model for *Z. marina* populations not previously described in the literature. The semi-annual life history strategy of *Z. marina* at NC1 was defined by seedlings reproducing both sexually and asexually before completing their life cycle in less than 12 months. The life history reported here ensures that a portion of the population at NC1 produces seeds during the first year of growth while also maximizing a successful clonal genotype through vegetative expansion. The development of the semi-annual life-history strategy at NC1 is not understood and further investigation into genetic variations and phenological response of this population to environmental conditions is required. Seed-bank viability was greater at the semi-annual site; however, seed-bank viability decreased over time with no viable seeds remaining after 6 months in the sediment. These results highlight the need for an annual replenishment of the seed-bank in both perennial and semi-annual

life history forms of *Z. marina* and indicate that the resiliency provided by the sediment seed-bank may be limited.

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Table 1-1 Sediment characteristics for both North Carolina sampling sites. All values are monthly mean values \pm S.E.

Site	NC1				NC2			
Date	Jul-07	Dec-07	Jun-08	Sep-08	Jul-07	Dec-07	Jun-08	Sep-08
% Organic	3.0 \pm 0.2	3.3 \pm 0.3	3.5 \pm 0.8	2.8 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.2	0.7 \pm 0.1	1.2 \pm 0.2
Nutrients								
NH ₃ um	14.7 \pm 2.9	8.9 \pm 2.9	42.1 \pm 17.0	21.3 \pm 7.5	20.4 \pm 6.4	12.7 \pm 0.8	15.2 \pm 1.6	14.3 \pm 2.4
PO ₄ um	0.9 \pm 0.1	0.8 \pm 0.2	0.9 \pm 0.3	0.2 \pm 0.1	1.5 \pm 0.5	0.6 \pm 0.2	3.8 \pm 1.2	0.0 \pm 0.0

Table 1-2. Results of repeated measures ANOVA for above-ground biomass and flowering shoot biomass for both sites over the 2007-2008 growing season. All significant results are denoted with an (*).

Factor	df	Type III SS	Mean SS	F value	<i>p</i>
<u>Vegetative Shoots</u>					
Between Sites					
Site	1	0.33	0.33	0.56	0.477
Error (site)	8	4.73	0.59		
Within Sites					
Time	15	214.55	14.30	23.20	<0.001*
Time*Site	15	45.24	3.02	4.89	<0.001*
Error (time)	120	73.98	0.62		
<u>Reproductive Shoots</u>					
Between Sites					
Site	1	2.65	2.65	17.52	0.003*
Error (site)	8	1.21	0.15		
Within Sites					
Time	4	3.23	0.81	10.10	<0.001*
Time*Site	4	0.83	0.21	2.59	0.055*
Error (time)	32	2.56	0.08		

Table 1-3. Negative binomial regression model analyzing the effects of site on vegetative shoot density, reproductive shoot density, potential number of produced seeds, total seed-bank density, and viable seed-bank density. Odds ratios calculated based on the parameter estimates. All significant results are denoted with an (*).

Parameter	DF	Est	SE	X ²	p	odds ratio	Wald 95 % CL	
							Low	High
<u>Vegetative Shoots</u>								
Intercept	1	4.28	0.08	2745.61	<0.001*	72.23		
Site	1	-0.90	0.12	56.95	<0.001*	0.41	0.32	0.51
Dispersion	1	0.47	0.06					
<u>Reproductive Shoots</u>								
Intercept	1	2.43	0.17	214.88	<0.001*	11.32		
Site	1	0.03	0.22	0.02	0.889	1.03	0.67	1.58
Dispersion	1	0.51	0.11					
<u>Potential Seed Production</u>								
Intercept	1	5.61	0.23	600.26	<0.001*	272.95		
Site	1	0.54	0.32	2.84	0.092	1.72	3.00	246.53
Dispersion	1	1.25	0.21					
<u>Seed-bank</u>								
Intercept	1	-0.28	0.18	2.52	0.112	0.75		
Site	1	1.22	0.23	29.78	<0.001*	3.42	2.20	5.31
Dispersion	1	0.86	0.21					
<u>Viable Seed-bank</u>								
Intercept	1	-4.23	1.05	16.14	<0.001*	0.01		
Site	1	3.30	1.13	8.62	0.003*	27.18	3.00	246.53
Dispersion	1	7.66	3.53					

Table 1-4. Mean sexual reproductive output for *Z. marina* beds located at both NC1 and NC2 during the 2007-2008 growing season. Values are averaged across all months when reproductive shoots were present (February to July). Values are reported as mean \pm S.E.

Sexual Reproductive Output	NC1	NC2
# reproductive shoots m ⁻²	1280 \pm 206	935 \pm 223
# rhipidia per shoot	3.5 \pm 0.2	3.5 \pm 0.4
# seeds per rhipidia	11 \pm 1	10 \pm 1
# seeds m ⁻²	53,513 \pm 7,365	36,000 \pm 8,595
% seed-bank retention	1.47 \pm 0.21	0.91 \pm 0.21

Table 1-5. Mean total seed-bank density and percentage of viable seeds for *Z. marina* beds in both NC1 and NC2. Values are mean \pm S.E.

Date	Total Density		% Viable	
	NC1	NC2	NC1	NC2
7/24/2007	190 \pm 97	211 \pm 74	33 \pm 21	13 \pm 13
12/28/2007	232 \pm 84	211 \pm 100	0 \pm 0	0 \pm 0
6/18/2008	906 \pm 246	147 \pm 42	18 \pm 9	0 \pm 0
9/15/2008	1243 \pm 147	147 \pm 79	10 \pm 4	0 \pm 0
10/31/2008	779 \pm 140	232 \pm 77	0 \pm 0	0 \pm 0
11/18/2008	232 \pm 91	147 \pm 26	0 \pm 0	0 \pm 0

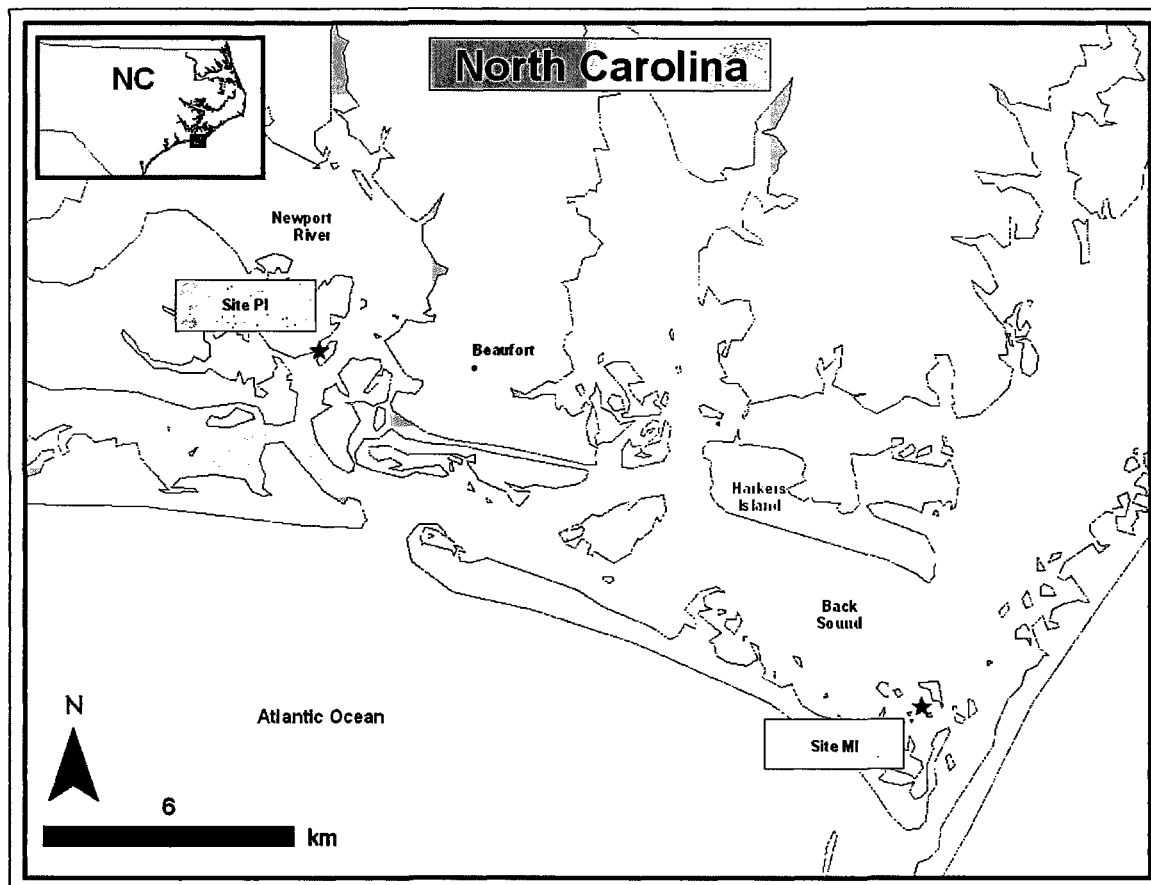


Figure 1-1. Location of NC1 and NC2 sampling sites in the southern outer banks region of North Carolina. Site locations are denoted with a star.

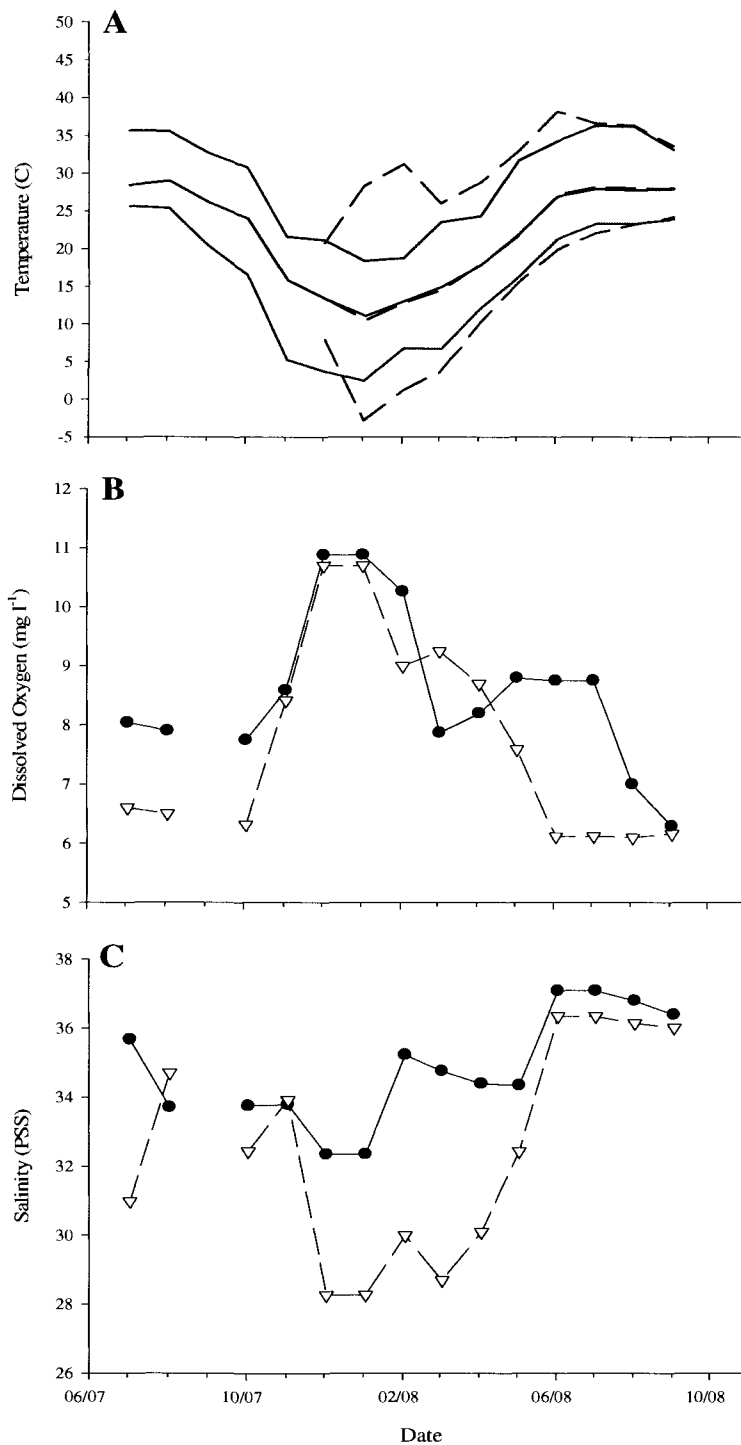


Figure 1-2. Mean water column (A) temperature, (B) DO, and (C) salinity for both sites over time. (A) Temperature data given as mean values (black) with minimum (blue) and maximum (red) values given for each month. (B) DO and (C) salinity values given as monthly averages. For all graphs NC1 data are given by dashed lines and NC2 data are denoted by solid lines.

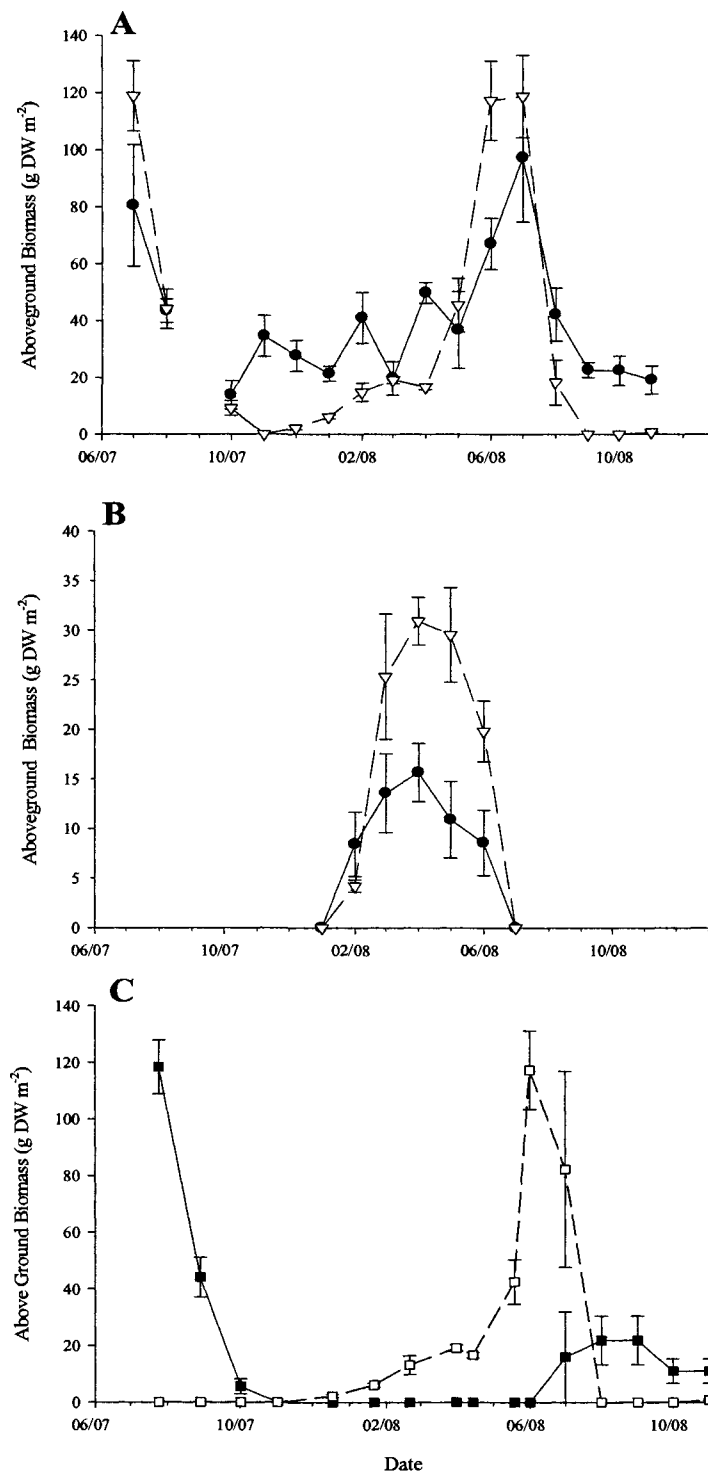


Figure 1-3. Mean \pm SE aboveground biomass for (A) non-reproductive shoots and (B) reproductive shoots during the 2007 – 2008 growing season at NC1 and NC2. NC1 = triangles. NC2 = circles. (C) NC1 2007 – 2008 surviving vegetative shoots and seedling above ground biomass. Surviving shoots = solid squares. Seedlings = open squares.

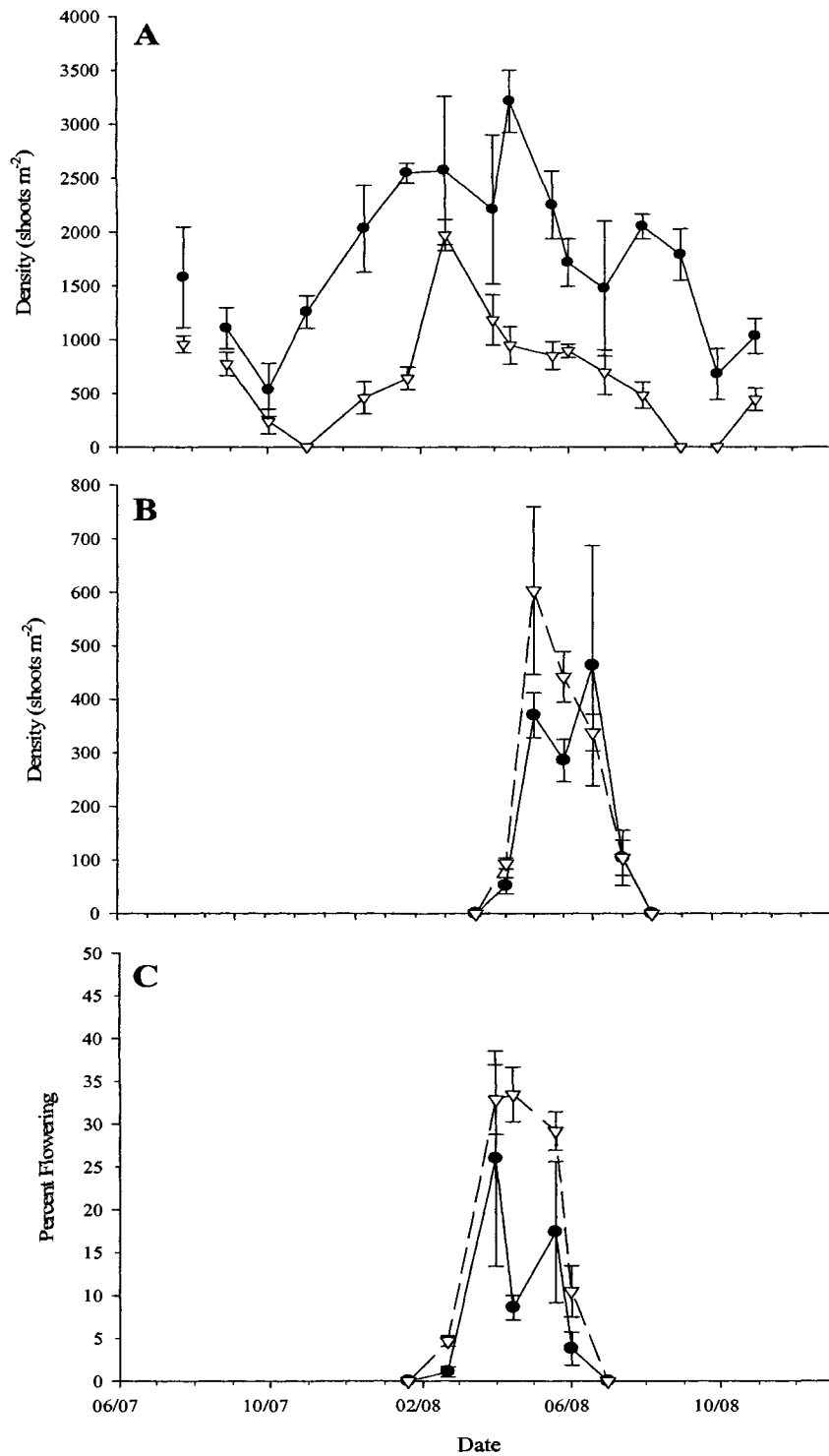


Figure 1-4. Mean monthly values \pm standard errors for (A) vegetative shoot density (B) reproductive shoot density and (C) percentage of reproductive shoots at both NC1 and NC2. NC1 data = triangles and dashed lines. NC2 data = circles with solid lines.

CHAPTER 2 : VIABILITY OF ZOSTERA MARINA L. SEMI-ANNUAL AND
PERENNIAL SEEDS IN THE SEDIMENT SEED-BANK

Abstract

A seed viability study quantified the effects of time (6, 12, 15 months), seed source (semi-annual, perennial – North Carolina; perennial – Virginia), site (sand substrate, mud substrate), and sediment type ($\geq 90\%$ sand, $\leq 80\%$ sand) on the viability of *Zostera marina* seeds in two separate experiments in semi-annual and perennial beds in the southern Albemarle-Pamlico Sound, North Carolina and in perennial beds in the lower Chesapeake Bay, Virginia in 2007 and 2008. In addition, the effect of time and site on ambient seed-bank viability was also quantified for all sites. Overall both North Carolina (NC) sites had greater total seed-bank densities (147 ± 79 to $1,243 \pm 147$ seeds m^{-2}) and greater seed-bank viability (0 to 33 ± 21 %) than the Virginia (VA) sites (maximum density 53 ± 18 seeds m^{-2} ; maximum viability 10 ± 10 %). Within the NC sites the semi-annual bed seed-bank density was 9.9 times greater and contained 3.3 times more viable seeds than the perennial bed. NC seed-bank densities followed *Z. marina* germination cycles and were the lowest following the period of maximum germination (October – November) in December 2007. VA ambient seed-bank densities were too low to quantify differences over time or between sites. Experimental results indicate that viability of NC seeds decreased significantly after 6 months in the sediment ($p < 0.001$). Site also had a significant effect on NC seed viability with 1.3 times more viable seeds collected from the muddy site compared to the sandy site ($p = 0.002$). Sediment type and seed source (semi-annual, perennial) did not have a significant effect on NC seed viability ($p = 1.000$ and 0.109 respectively). As with the NC seeds, viability of VA seeds decreased with time ($p < 0.001$). Viability of VA seeds decreased from 42 % to 0 – 4 % after 6 months in the sediment. There was no direct effect of seed source (perennial sand, perennial

mud) on experimental VA seed viability ($p = 1.000$). Based on the experimental results and ambient seed-bank monitoring, time was the over riding factor affecting both semi-annual and perennial *Z. marina* seed viability in both NC and VA populations, resulting in the production of transient seed-banks at all sites. The significant reduction of seed viability after only 6 months in the sediment suggests that the resiliency provided by seed-banks for both perennial and semi-annual *Z. marina* beds may be limited by seed production on annual scales and by timing of disturbance events.

Key Words: seagrass, *Zostera marina*, semi-annual, seed-bank, viability

Introduction

Zostera marina is a dominant seagrass species circumglobally distributed in temperate coastal environments in the Northern Hemisphere (den Hartog, 1970; Green and Short, 2003; Short and Moore, 2006). Although found in both perennial, annual, and semi-annual forms, the majority of *Z. marina* beds are perennial and maintain their populations primarily through clonal expansion and vegetative growth (Setchell, 1929; den Hartog, 1970; Tomlinson, 1974; Hemminga and Duarte, 2000; Rasheed, 2004; Jarvis Chapter 1). Despite the prevalence of asexual reproduction, all *Z. marina* populations retain the ability to flower (Setchell, 1929; den Hartog, 1970; Thayer et al., 1984). Within *Z. marina* populations, sexual reproduction increases genetic diversity (Ackerman et al., 2006), enhances recovery from small and large-scale declines (Plus et al., 2003; Whitfield et al., 2004; Waycott et al., 2005), and provides a mechanism for increasing the geographic range and establishment of new meadows (Figuerola et al., 2002; Källström et al., 2008). The ability to re-establish populations and expand into new suitable habitats has become increasingly important over the last several decades as seagrass populations, including *Z. marina*, are declining on a global scale in response to anthropogenic disturbances (Short and Wyllie Echeverria, 1996).

Z. marina has developed three life-history strategies to fully exploit habitats ranging from tide pools and exposed intertidal mud flats to deeper subtidal light limited areas (Keddy and Patriquin, 1978; Phillips et al., 1983a; Robertson and Mann, 1984; van Lent and Verschuure, 1994; Jarvis Chapter 1). The current perennial life-history model for *Z. marina* contains both asexual and sexual reproduction with changes in growth and reproduction dictated by water temperature (Setchell, 1929; Phillips et al., 1983;

Silberhorn et al., 1983; Thayer et al., 1984; Short and Moore, 2006). Although perennial *Z. marina* populations increase sexual reproduction in response to disturbances (Phillips et al., 1983; den Hartog, 1987; van Lent and Verschuure, 1994), the current model emphasizes clonal expansion and does not take the seed-bank, seed germination, or seedling growth and survival into account (Setchell, 1929; Thayer et al., 1984). The annual life-history strategy for *Z. marina* is reliant completely on sexual reproduction with no clonal growth (Keddy and Patriquin, 1978; Phillips et al., 1983a; Robertson and Mann, 1984; Santamaría-Gallegos et al., 2000). In addition, annual populations have a compressed life cycle with seedlings germinating, flowering, producing seeds and dying in less than a year (Keddy and Patriquin, 1978; Phillips et al., 1983a; Santamaría-Gallegos et al., 2000). Seeds remain within the sediment seed-bank until germination occurs the following year (Keddy and Patriquin, 1978; Phillips et al., 1983). Like annual populations, semi-annual *Z. marina* populations rely on sexual reproduction for bed re-establishment on an annual basis, flower as seedlings, and complete their life cycle in less than one year; however, they also reproduce asexually (Jarvis, Chapter 1).

Flowering within *Z. marina* beds is not homogeneous, resulting in a non-even distribution of seeds within the sediment seed-bank (Harwell and Orth, 2002; Morita et al., 2007). Annual and perennial *Zostera marina* seeds typically do not germinate immediately after release from the reproductive shoot (Taylor, 1957; Keddy, 1987; Harrison, 1993; Moore et al., 1993). Once deposited on the sediment surface seeds may become incorporated into the sediment forming a seed-bank (Simpson, 1990; Harwell and Orth, 2002). Sediment seed-banks are defined as a reservoir of seeds found within the sediment which are viable, or capable of germinating, and therefore are able to

replace adult plants (Baker, 1989). Seagrass seed-banks provide resiliency from small and large scale declines (Olsen and Sand-Jensen, 1994; Plus et al., 2003; Olsen et al., 2004; Greve et al., 2005), a mechanism of re-establishment for annual species (Hootsmans et al., 1987; Hammerstrom et al., 2006; Lee et al., 2007), and a mode of dispersal within the sediment (Bell et al., 2008). Although *Z. marina* seeds are not produced at or below the sediment surface, which for seagrass genera *Halodule*, *Cymodocea*, and *Halophila* increases the probability for incorporation of seeds into the sediment seed-bank (Inglis, 2000), seeds are negatively buoyant and do not move more than a few meters once deposited onto the sediment (Orth et al., 1994). Despite the lack of direct burial into the sediment, perennial *Z. marina* seed-banks have been reported to contain between 0 and 1,200 seeds m⁻² and annual seed-banks between 1,300 to 30,000 seeds m⁻² (Harrison, 1993; Harwell and Orth, 2002; Lee et al., 2007; Mortia et al., 2007).

For the sediment seed-bank to perform any function, seeds have to be maintained at the correct physiological state to ensure viability while also remaining in an environment conducive for germination to occur at the necessary time (Murdoch and Ellis, 2000; Thompson, 2000). Seed viability can be affected by many biotic (i.e. predation) and abiotic (i.e. burial depth, sediment type, temperature, salinity) factors in the surrounding environment (Baskin and Baskin, 1998; Murdoch and Ellis, 2000). Seed-banks that retain viable seeds for less than one year are classified as transient while seed-banks that retain viable seeds for more than one year are persistent (Thompson and Grime, 1979). Under laboratory conditions, *Z. marina* seeds do not remain viable in a liquid medium for longer than 11 months (McMillan, 1983; Harrison, 1991; Moore et al., 1993) and therefore are assumed to produce transient seed-banks (Harwell and Orth,

2002). The micro-environment created by sediment conditions may have a significant affect on the longevity of seed viability and may affect the type (transient or persistent) of sediment seed-bank produced (Harper, 1977). However, there has been little research directly testing the effects of time and variations in the surrounding sediment environment on seed-bank viability. Understanding the effects of time and sediment conditions on long term seed-bank viability is necessary to begin quantifying the role of seeds in the maintenance and recovery of seagrass populations.

Z. marina populations in the lower Chesapeake Bay, Virginia and in the Newport River and Back Sound, North Carolina were selected to quantify the effects of time and sediment conditions on seed-bank viability due to stressful environmental conditions associated with populations found at the species geographic limit (Kenworthy et al., 1981; Keddy and Patriquin, 1978; Phillips et al., 1983; Santamaría-Gallegos et al., 2000). *Z. marina* growth and survival at these sites, which are located at the southern limit of the species distribution along the western Atlantic (Thayer et al. 1984, Short et al., 2007), can be limited in the late summer when water temperatures exceed 30 °C (Setchell, 1929; Thayer et al., 1984). In both sites water temperatures have increased more than 1.0 °C over the last fifty years (Preston, 2004; Micheli et al., 2008) and are predicted to continue rising over the next several decades (Harley et al., 2006). By quantifying the effects of sediment composition and time on *Z. marina* seed-bank viability the resiliency provided by the sediment seed-bank to increasing stressful environmental conditions may also be quantified.

The purpose of this study was to use field experiments to quantify the *in situ* effects of seed source (North Carolina – semi-annual, perennial; Virginia – perennial

only), time (6, 12, 15 months), sediment type (sand, mud) and site on *Z. marina* seed viability between semi-annual and perennial beds in the Newport River/Back Sound, Carteret County, North Carolina and in a separate experiment among perennial beds in Chesapeake Bay, Virginia. In addition, the density and viability of ambient sediment seed-banks was monitored over a 15 month period at all sites. I hypothesized that (1) seeds derived from a mixed life history strategy bed would not have greater viability than seeds derived from a perennial bed, (2) seed viability would not be significantly different between sediments and sites similar to those found in the seed source bed (i.e. seeds collected from sandy sites would maintain similar viability in either sandy or muddy sediments and at sandy or muddy sites), and (3) overall seed viability would not decrease over time.

Methods

Site Selection

The effect of sediment type (sand, mud), seed source, (semi-annual strategy and perennial strategy), site (sandy substrate, muddy substrate), and time (0, 6, 12, 15 months) on the viability of *Z. marina* seed-banks was quantified in two separate field experiments in the Newport River/Back Sound, North Carolina and in the lower Chesapeake Bay, Virginia in 2007 and 2008. Experimental sites were selected in North Carolina based on the historical presence of perennial and semi-annual beds of *Z. marina* (Thayer et al., 1984) and preliminary sediment organic content and grain size analyses. Virginia experimental sites were selected based on historical presence of perennial *Z. marina* beds (Orth et al., 2006) and sediment composition. Seeds and sediment for the

North Carolina experiment were collected from Phillips Island (NC1, 34° 43' N, 76° 41' W), an semi-annual *Z. marina* bed with a muddy substrate ($\geq 90\%$ sand) in the Newport River and from Morgans Island (NC2, 34° 66' N, 76° 52' W) a perennial bed with a sandy substrate ($\leq 80\%$ sand) in Back Sound (Figure 2-1). For the Virginia experiment two historically persistent perennial beds in the lower York River, a tributary to the Chesapeake Bay, were selected at the muddy Allens Island site (CB1, 37° 15' N; 76° 25' W) and at the sandy Goodwin Island site (CB2, 37° 13' N; 76° 23' W; Figure 2-1).

Seed Collection and Seed Viability

Reproductive shoots were collected from all four *Z. marina* beds during the period of maximum seed release; early to late May in North Carolina (Thayer et al., 1984) and late May and early June in Chesapeake Bay (Silberhorn et al., 1983).

Reproductive shoots were kept in separate aerated flow-through seawater tanks (2.4 m x 1.2 m x 1.0 m) until seeds dehisced from the reproductive shoots approximately one month later (Orth et al., 2007). The seeds were then collected from the bottom of the tank and kept in separate containers in aerated recirculating tanks (2.4 m x 1.2 m x 1.0 m) at 20 °C for one month until placement in experimental cores.

A sub-sample of 100 seeds from each seed source was tested for viability using tetrazolium chloride (Lakon, 1949; AOSA 1981; Conacher et al., 1994; Sawma and Mohler, 2002) prior to both experiments. Tetrazolium chloride was used due to increased accuracy and greater time efficiency compared to traditional germination tests (Lakon, 1949; AOSA 1981; Sawma and Mohler, 2002). Seed embryos were removed from their seed coats and soaked in a 1 % tetrazolium chloride solution for 24 hours before

examination on a dissecting scope at 10 x magnification (Conacher et al., 1994). Seeds with a pink to brown stained cotyledon and axial hypocotyl were considered viable (Taylor, 1957; Harrison, 1993).

Seed Viability Experiments

Sediment Collection and Characterization

Sediment for all experimental treatment cores was collected from each site and stored at 7 to 9 °C until processing. Prior to analysis all sediment was dry sieved (0.5 mm mesh) to remove any extraneous seeds and then homogenized. Five sediment plugs (10.4 cm diameter by 3 cm depth) were collected from each sediment type and quartered. The first quarter was wet sieved (63 µm sieve), washing slit and clay fractions into a graduated cylinder. After 24 hours, pipette analysis was performed with the filtrate to determine the clay (8 phi) and silt (4 phi) fractions of the sieved samples (modification of Plumb, 1981). Dry weights of the aliquots were then compared and percent sand silt and clay fractions were determined.

After dry sieving to remove extraneous seeds, sediment percent organic matter was determined by drying one half of the sediment sub-sample at 60 °C for 5 days or until a constant dry weight was reached. The samples were cooled in a desiccator and 10 g of sediment was weighed and combusted at 500 °C for five hours. The sample was weighed again and percent organic matter calculated (Erftemeijer and Koch, 2001). Sediment exchangeable nutrients of the remaining quarter of the sample were extracted in 2 M KCl, shaken for 1 hour, centrifuged at 4000 RPM for 6 minutes, filtered (Gelman Supor, 0.45 µm), and frozen until analysis. Dissolved inorganic nitrogen (DIN, NH_4^+

only) and dissolved inorganic phosphate (DIP, PO_4^{-3}) were determined using a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002).

North Carolina Seed Viability and Sediment Cores

Experimental seed viability cores (10.2 cm diameter x 15.2 cm) were partially filled with sieved sediment and 50 seeds from each seed source were placed at depths between 3 and 6 cm. Seed depth was selected based on observed vertical distributions of viable *Z. marina* seeds in established seed-banks (Harrison, 1991; Harwell and Orth, 2002). Once the seeds were planted, the cores were capped with sediment and covered with plastic mesh screening (0.5 cm) on both ends. Experimental treatments at each site consisted of seeds derived from the semi-annual population placed in sand sediments and in mud sediments, and seeds derived from the perennial population placed in sand sediments and in mud sediments.

In addition, supplementary experimental sediment cores identical to the viability cores, but filled with sediment only (no seeds), were established to quantify potential experimental artifacts on sediment conditions within the viability cores. These cores were necessary due to the destructive sampling protocols for sediment pore water nutrients and organic content. All cores (treatment and supplementary sediment) were replicated three times for each sampling period (total = 12 treatment cores and 6 sediment cores per site per sampling period).

Three 1 m² plots were established in July 2007 within vegetated areas at both NC1 and NC2. Within each plot, one replicate core for each treatment was buried flush

with the sediment surface in randomly selected 20 cm² quadrats within the plots. For the North Carolina experiment, one replicate treatment core and one supplementary sediment core for each sediment type were removed at each sampling time: 6 months (December 2007), 12 months (June 2008), and 15 months (September 2008) (18 cores per sampling month per site).

Virginia Seed Viability and Sediment Cores

Virginia treatments and sediment cores were identical to the North Carolina cores except that sediment and seeds were collected from *Z. marina* beds in the lower York River. The Virginia experimental treatments also compared the effects of seed source, sediment type, site, and time on sediment seed-bank viability. However, Virginia seed source treatments did not differ in reproductive strategy as both sources were derived from known perennial populations. Seeds collected from CB1 were exposed to a site characterized by sandy sediments (Buzzelli, 1998) where seeds from CB2 were collected from a site characterized by silt-clay dominated sediments (Hobbs, 1994; Jarvis Chapter 4). Experimental treatments in the Virginia cores consisted of perennial seeds collected from sandy sites in sand sediments, perennial seeds collected from sandy sites in mud sediments, perennial seeds from muddy sites in sand sediments, and perennial seeds from muddy sites in mud sediments. As with the North Carolina sites, three 2 m² plots were established at each Virginia site and one replicate set of treatment and sediment cores were collected after 6 months (January 2008), 12 months (June 2008), and 15 months (September 2008).

Treatment Core Collection and Ambient Seed-bank Characterization

All collected experimental cores were wet sieved (0.5 mm mesh) to remove all sediment. Seeds (intact seed coat) and seed coats (seed coat split open with missing embryo) were collected and counted. Intact seeds were tested for viability using tetrazolium staining as described previously.

To quantify the total density and viability of the ambient seed-bank, five additional cores (10 cm diameter, 10 cm length) were also collected within a 2 m² area around the plots at each site. Ambient seed-bank cores were processed identically to the treatment cores. Total seed density, viability of the seed-bank, and the changes in seed viability were quantified.

Ambient Site Characterization

To characterize ambient sediments at each site six additional ambient sediment cores (4 cm diameter, 10 cm length) were collected from within a 2 m area immediately surrounding each plot. Day time vertical redox (Eh) profiles were quantified for the remaining experimental and sediment cores with a 21 cm platinum electrode. The probe was inserted into the top of the core and redox was measured at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 cm. Final readings were corrected for temperature relative to the reference electrode (Hinchey and Schaffner, 2005). Following redox analysis, each experimental sediment only core and three of the ambient sediment cores were cut into sections at 3 cm and 6 cm depths all sections were then halved. The first section was analyzed for organic content and the second half was analyzed for sediment exchangeable

nutrients as described previously. Although samples were collected during each sampling date, percent sand/silt/clay analysis was only completed on the initial samples.

Water temperature ($^{\circ}\text{C}$), salinity (PSS), and dissolved oxygen (mg l^{-1}) were measured with a Yellow Spring Instruments, Inc. (YSI, Inc., Yellow Spring, Ohio) model 650 sonde at each site during each core sampling. In addition, three water samples were collected, filtered (Gelman Supor, $0.45\ \mu\text{m}$), and frozen until analyzed for DIN and DIP with a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002). The remaining water samples were filtered and analyzed for chlorophyll *a* (Strickland and Parson, 1972) and total suspended solids (TSS). TSS was quantified from a well-mixed sample of known volume. The sample was filtered through a GF/F filter and the residue retained on the filter was dried to constant weight at $103 - 105^{\circ}\text{C}$ and reported as $\text{mg total suspended solids l}^{-1}$.

Data Analysis

Prior to the beginning of the experiments a set of models was developed to describe the relationship between seed viability, seed source, time, sediment type, and site (Table 2-1). In addition, a second set of models was derived to describe the relationship between time, site and the total density and the proportion of viable of seeds in the ambient, sediment seed-bank at each site (Table 2-1). The models were used for both North Carolina and Virginia treatments, although all experimental and sediment seed-bank data were analyzed separately. For all analyses it was assumed that viability of one seed did not significantly affect the viability of surrounding seeds (Orth et al.,

2003). Therefore, each seed was considered an independent Bernoulli trial and analyzed separately.

Experimental core seed viability data were analyzed using logistic regression with time, seed source, sediment type, and site as factors (PROC GENMOD; SAS Institute Inc.; Scott et al., 1984). Logistic regression was selected due to the binary response variable, the large number of observations (600 per treatment), and low number of viable seeds throughout the course of the experiment. Site seed-bank viability was also analyzed using logistic regression with time and site as factors.

To compare differences between the total numbers of seeds in the sediment seed-bank at each site, the data were analyzed using negative binomial regression with time and site as factors (PROC GENMOD; SAS Institute Inc.; Allison, 1999). Negative binomial regression was selected due to the discrete highly skewed nature of the ambient seed-bank data for all sites (Allison, 1999).

To determine the best fitting model the second-order Akaike Information Criterion (AIC_c) was calculated using log likelihood ratios derived from all regression analyses (Burnham and Anderson, 2002). AIC_c differences (Δ_i) between all models were then calculated and the models were ranked. The model with the smallest Δ_i was selected as the best fitting model. For all significant ($p < 0.05$) model terms, odds ratios were calculated using Wald chi square statistics (SAS; SAS Institute Inc). Likelihood ratio tests for all parameter estimates were also calculated and compared to the Wald Chi Square Statistics (Allison, 1999).

Differences in sediment organic content and sediment exchangeable nutrients between sediment types, sites, and over time were analyzed with nonparametric statistics

as the data were non-normally distributed (Kruskal-Wallis and Kolmogorov-Smirnov Tests; The SAS System for Windows, SAS Institute Inc.). A three-way Analysis of Variance (ANOVA) was used to compare the effects of time, sediment type, site and the interactions of these factors on redox measurements. All *post hoc* analyses of the data were performed with Tukey's HSD test (Tukey's Test; The SAS System for Windows, SAS Institute Inc.).

Results

Sediment Characteristics

For both the North Carolina and Virginia site characterizations, all sediment characterization data, except for redox measurements, were averaged across dates and depths and presented as mean values per site \pm standard errors (Table 2-2).

North Carolina Ambient Sediment Characteristics

Site was the only factor that significantly affected ambient sediment organic matter ($p < 0.001$), ambient porewater dissolved inorganic nitrogen concentrations ($p = 0.031$), and ambient sand/silt/clay ratios ($p = 0.005$) in North Carolina sediments. Sediment percent organic matter concentrations, NH_4^+ concentrations, and silt/clay ratios were significantly greater at NC1 than NC2. However, PO_4^{-3} concentrations did not differ significantly with site ($p = 0.446$). Ambient organic content, porewater nutrients, and percent sand, silt, and clay did not differ significantly with depth (% organic $p = 0.999$; $\text{NH}_4^+ = 0.260$; $\text{PO}_4^{-3} = 0.260$; sand, silt, and clay $p = 0.446$) or over time (% organic $p = 0.790$; $\text{NH}_4^+ = 0.453$; $\text{PO}_4^{-3} p = 0.470$) at either North Carolina site. North

Carolina site sediment redox profiles did not differ significantly between sites ($p = 0.060$) or over time ($p = 0.071$) but there was a significant difference with depth ($p = 0.014$). *Post hoc* analysis with Tukeys HSD test indicated a significant difference in redox levels between 0 cm and all other depths; between 0.5 cm and depths greater than 1.5 cm; and between 1.0 cm and depths greater than 3.0 cm for both sites (Figure 2-2). Redox levels were more reduced with depth until 3 cm where they became constant to a depth of 10 cm.

Virginia Ambient Sediment Characteristics

Virginia site sediments showed similar trends to the North Carolina sites with ambient sediment organic content ($p < 0.001$), sediment nutrients (NH_3^+ $p < 0.001$; PO_4^{-3} $p = 0.019$), and sand/silt/clay ratios ($p = 0.005$) all significantly affected by site. Organic content, NH_3^+ concentrations, and silt clay ratios were greater at CB than CB2 (Table 2-2). In addition, Virginia site percent organic matter and sediment exchangeable nutrient concentrations did not differ significantly with depth (% organic $p = 0.999$; NH_3^+ $p = 0.446$; PO_4^- $p = 0.774$) or over time (% organic $p = 0.901$; NH_3^+ $p = 0.303$). Virginia sediment PO_4^{-3} did differ significantly with date ($p = 0.001$) however levels were near 0 μM . For the Virginia sites redox profiles were significantly different between sites ($p < 0.001$) and dates ($p < 0.001$). There was also a significant site by date interaction ($p < 0.001$). Redox conditions were more reduced during the summer sampling periods at CB1 compared to CB2 (Figure 2-2). While redox was not significantly different between depths ($p = 0.077$) *post hoc* analysis with Tukey's HSD test indicates that redox

values were significantly more reduced with depth to a depth of 3 cm after which they stabilized.

North Carolina Experimental Core Sediments

As expected, sediment percent organic matter in the experimental cores differed significantly between sediment types ($p < 0.001$) and between sites ($p = 0.011$). Percent organic matter did not differ significantly with depth ($p = 0.271$) or between sampling dates ($p = 0.235$). The difference in organic matter between sites was a result of higher organic matter concentrations in the sand treatment at NC1 ($2.27 \pm 0.34 \%$) compared to the same treatment at NC2 ($1.31 \pm 0.10\%$; Table 2-2). There was no significant difference in organic matter between sites for the mud treatments.

Sediment redox profiles were also significantly affected by site ($p < 0.001$) and sediment type ($p < 0.001$) as sediments were more reduced in both sand and mud treatments at NC1 compared to NC2 (Figure 2-3). In addition, redox values were significantly different over time ($p = 0.038$). For all treatments except for the sand treatment at NC1, higher redox values were recorded at the 12 month sampling (December 2007) when water temperatures were at their lowest (Figure 2-3). Depth did not significantly affect redox values ($p = 0.212$). The overall sand/silt/clay ratios were similar between sand and mud treatments at each site ($p = 0.482$). There was also no significant difference in sediment exchangeable porewater nutrients between sites or sediment treatments (NH_3^+ $p = 0.322$; PO_4^{3-} $p = 0.105$).

Virginia Experimental Core Sediments

Sediment percent organic matter in the experimental cores differed significantly between sediment types ($p < 0.001$) but did not differ significantly between sites ($p = 0.079$) with depth ($p = 0.810$) or between sampling dates ($p = 0.654$). Sediment redox profiles were also significantly affected by site ($p < 0.001$) and sediment type ($p < 0.001$) as sediments were more reduced in both sand and mud treatments at CB1 compared to CB2 (Figure 2-4). In addition, redox values were significantly different over time ($p < 0.001$) and depth ($p < 0.001$) and there were significant time and site interactions ($p < 0.001$) and time depth interactions ($p < 0.001$). Redox values were significantly different between all sampling dates with the most reduced values recorded in August 2007 (Figure 2-4). Surface redox values (0 cm) were significantly less reduced than all sediment redox values. Redox recorded at depths greater than 3 cm were significantly lower than redox values between 0.5 and 2 cm.

The percentage of clay in the sediments was similar between sites ($p = 0.461$); however percent sand was significantly greater at CB2 ($p = 0.032$) and percent silt was significantly greater at CB1 ($p = 0.032$). Sediment exchangeable porewater PO_4^{-3} was significantly greater ($p = 0.040$) in CB1 core sediments than CB2; however for both sites PO_4^{-3} values were less than $1 \mu\text{M}$ (Table 2-2). There was no significant difference between sediment porewater NH_3^+ ($p = 0.959$) between sites. There was also no significant difference in sediment exchangeable porewater nutrients between sediment treatments (NH_3^+ $p = 0.305$; PO_4^{-3} $p = 0.999$).

Ambient Seed-bank Viability

North Carolina Ambient Seed-bank

Throughout the course of monitoring the North Carolina sites total seed-bank densities ranged from 147 ± 79 to $1,243 \pm 147$ seeds m^{-2} in the ambient sediments (Table 2-3). Based on differences between AIC_c values, there were two models that were equal in describing the relationship between total seed density and the factors of time and site (Table 2-4). The results of the top ranked equation (V_3) indicate that seed-bank viability was not significantly affected by time (likelihood ratio chi square $p = 0.469$), but was significantly affected by site ($p < 0.001$; Table 2-5). Although there was no significant effect of time on total seed-bank density, the odds ratios calculated from the parameter estimates indicate that there were 1.2 times fewer seeds in the seed-bank in December 2007 than in July 2007, 1.4 less than in June 2008, and 1.8 times less than in September 2008 (Table 2-5). Site had the greatest impact on total seed-bank density with 3.4 times more seeds at NC1 compared to NC2.

The model results also indicate that overall North Carolina seed-bank viability was not significantly affected by time (12 months $p = 0.520$; 15 months $p = 0.182$; Table 2-5). However, no viable seeds were collected during the 6 month sampling in December 2007 from either site. As a result, the logistic regression did not compare seed viability at 6 months to initial viability samples. The likelihood chi square estimate was able to control for the highly skewed data and indicated that time was highly significant ($p = 0.001$). Overall the greatest number of viable seeds in the seed-bank occurred in September 2008. The seed-bank at NC1 had 9.9 times more viable seeds than the seed-bank at NC2 (Table 2-3).

V_3 was selected as the representative equation to describe total seed-bank density in North Carolina (Table 2-4). While this equation does not take the effects of time into account, the effects of site on total seed-bank density were also highly significant ($p < 0.001$; Table 2-5). Odds ratios indicate that there were 3.6 times more seeds in the NC1 sediment seed-bank compared to the NC2 seed-bank.

Virginia Ambient Seed-bank

The ambient seed-bank in both Virginia sites was severely depleted through out the entire monitoring period (Table 2-3). No seeds were found in either site in the August 2007, January 2008, and July 2008 samples. Seeds were only found in the CB1 sediment seed-bank in October of 2008. Between all cores collected at both Virginia sites 10 seeds total were collected. Based on the extremely low number of seeds collected from the ambient sediment seed-bank, no analysis could be done on either total seed densities or on seed-bank viability.

Seed Viability Experiments

North Carolina Experimental Seed Cores

North Carolina seed viability was best described by the relationship among time, site, sediment, seed source and the individual interactions between time and all other factors (V_{15} ; Table 2-3). For all experimental seed viability data, site refers only to the location of the cores. Viability of North Carolina seeds in the experimental cores decreased significantly with increasing time in the sediment (likelihood chi square estimate $p < 0.001$; Figure 2-5). The effect of time on the density of viable seeds

emerged by the 6 month sampling in December 2007 when there were 160 times more non-viable seeds compared to initial seed viabilities ($p < 0.001$; Table 2-6). After the first initial decline in viability, the trend decreased with only 2.4 times more viable seeds found at 6 months compared to the 12 month sampling. The trend decreased even further between 6 months and 15 months with only 1.5 times more viable seeds found at 6 months (Figure 2-5). Site also had a significant effect of viability on experimental seed density with 1.3 times more viable seeds collected from cores placed in NC1 compared to NC2 ($p = 0.002$; Table 2-6).

While sediment (sandy, muddy) and seed source (semi-annual, perennial) were included in the model, there was no significant effect of either parameter on overall seed viability ($p = 1.000$ and 0.109 respectively; Table 2-6) in the experimental cores. There were significant interactions between time and both sediment type ($p < 0.001$) and seed source ($p = 0.013$; Table 2-6). There was a significant interaction between viability of seeds at 6 months and sediment type resulting from 6.2 times more viable seeds in muddy sediments than sandy sediments during this sampling (Figures 2-5 and 2-6). The source time interaction also occurred with the 6 month sampling. After 6 months in the sediment there were 2.6 times more non-viable semi-annual than perennial seeds remaining in the experimental cores. This significant trend did not continue during the 12 and 15 month sampling dates. During the course of the experiment, the proportion of unaccounted for or lost seeds had an inverse relationship with seed viability (Figure 2-6). As time elapsed from 0 to 6, 12, and 15 months the percentage of lost seeds also increased from 0 % of total seeds to 61 ± 10 % by 15 months for mud treatments and from 0 % to 68 ± 5 % for sand treatments

Virginia Experimental Seed Cores

During the model selection process for the Virginia experimental seed-core viability the top three models were rejected based on the negative of the Hessian not being definitive. Therefore, the top four models reported here do not include those which did not meet all requirements of the logistic regression model. The model used in analysis of experimental core data included the parameters time, seed source, and the interaction between time and seed source (Table 2-4). Sediment type and site were not included in the top ranked model and therefore, were not included in any further analysis.

As with the North Carolina seeds, viability of Virginia seeds decreased with increasing time in the sediment (likelihood ratio estimate $p < 0.001$; Figures 2-5 and 2-7). Although there was no significant difference between seed viability at 6 months and initial seed viabilities ($p = 0.254$; Table 2-6) seed viability had decreased from initial viabilities of 42 % to 4 % by 6 months. The lack of significance may be a reflection of the 0 % viability of seeds at 12 and 15 months. By 12 months seed viability decreased significantly ($p < 0.001$) resulting in 86 times more non-viable seeds in the sediment cores after 12 months compared to initial testing (Table 2-5). After 15 months, the number of viable seeds in the experimental cores was low enough and the data were skewed so that the odds ratio could not be calculated, although seed viability was significantly lower compared to initial seed viability ($p < 0.001$).

Although seed source was included in the model, there was no significant effect of seed source on experimental seed viability ($p = 1.000$; Table 2-5). There was a significant interaction between time and seed source on seed viability ($p < 0.001$). Seeds collected from CB2 (the high energy sandy site) were 13.8 times more viable than seeds

collected from the CB1 (the low energy muddy site) after 6 months in the sediment (Figure 2-4), but this trend did not hold throughout the entire experiment (Figures 2-5 and 2-7).

The proportion of unaccounted for or lost seeds in the Virginia experiment had an inverse relationship with seed viability (Figure 2-7). As time increased, the percentage of lost seeds increased from 0 % of total seeds to 38 ± 15 % by 15 months for mud treatments and from 0 % to 30 ± 10 % for sand treatments.

Water Column Characteristics

Water column conditions were similar at the two North Carolina sites with temperatures ranging from 7.2 ± 0.1 °C to 30.3 ± 0.1 °C at NC1 and from 4.8 ± 0.1 °C to 29.8 ± 0.1 °C at NC2. Water column chlorophyll *a* mean concentrations were less than $6.0 \mu\text{g l}^{-1}$ at both sites throughout 2007 and 2008. TSS followed a similar pattern to chlorophyll with concentrations averaging $< 30.0 \text{ mg l}^{-1}$ for both sites throughout the sampling period. Water column NH_3^+ concentrations ranged from $0.55 \pm 0.15 \mu\text{M}$ to $1.04 \pm 0.10 \mu\text{M}$ at NC1 and from $0.52 \pm 0.06 \mu\text{M}$ to $2.18 \pm 0.05 \mu\text{M}$ at NC2. Water column PO_4^{-3} concentrations ranged from $0.08 \pm 0.00 \mu\text{M}$ to $0.23 \pm 0.02 \mu\text{M}$ at NC1 and from $0.25 \pm 0.05 \mu\text{M}$ to $0.49 \pm 0.15 \mu\text{M}$ at NC2.

Water column conditions were also similar at the two sites in Virginia with water temperatures ranging from 0.89 °C to 32.39 °C in CB2 and 1.4 °C to 31.6 °C at CB1. Water column chlorophyll *a* ranged from $13.52 \pm 0.32 \mu\text{g l}^{-1}$ to $26.80 \pm 5.89 \mu\text{g l}^{-1}$ CB1 and from $6.07 \pm 0.06 \mu\text{g l}^{-1}$ to $23.55 \pm 0.92 \mu\text{g l}^{-1}$ at CB2. Total suspended solids ranged from $11.11 \pm 0.28 \text{ mg l}^{-1}$ to $23.00 \pm 7.27 \text{ mg l}^{-1}$ at CB1 and from $7.11 \pm 0.22 \text{ mg l}^{-1}$ to

$38.98 \pm 7.41 \text{ mg l}^{-1}$ at CB2. Water column nutrients were generally low at both sites. Concentrations of NH_3^+ ranged from $0.24 \pm 0.02 \text{ }\mu\text{M}$ to $4.92 \pm 0.79 \text{ }\mu\text{M}$ at CB2 and from $0.80 \pm 0.05 \text{ }\mu\text{M}$ to $4.79 \pm 0.45 \text{ }\mu\text{M}$ at CB1. Water column PO_4^- concentrations ranged from $0 \pm 0 \text{ }\mu\text{M}$ to $0.36 \pm 0.01 \text{ }\mu\text{M}$ at CB2 and from $0.21 \pm 0.02 \text{ }\mu\text{M}$ to $0.39 \pm 0.09 \text{ }\mu\text{M}$ at CB1.

Discussion

Based on the results of the *in situ* experiments and ambient seed-bank monitoring, semi-annual and perennial *Zostera marina* beds in North Carolina and perennial beds in Virginia produce transient (seeds remain viable less than 12 months in the sediment) seed-banks. Viability of all seed sources in both the Virginia and North Carolina *in situ* core experiments were reduced significantly after only 6 months in the sediment and were further reduced to < 5 % of initial viability by 15 months. These results are similar to the trends in the ambient data where both semi-annual and perennial NC ambient seed-bank viability decreased from 10 to 33 % depending on site in July 2007 to 0 % for all sites in December 2007. The loss of seed viability monitored for all seed sources and recorded at all sites presented here support experimental laboratory results which concluded that *Z. marina* seeds do not remain viable in water for longer than 11 months (McMillan, 1983; Harrison, 1991; Moore et al., 1993). Therefore, the loss of viability of perennial and semi-annual *Z. marina* seeds appears similar in both water and sedimentary environments.

Time was the only factor that significantly affected seed viability in both North Carolina and Virginia seeds in both the *in situ* field experiments and in the ambient seed-

bank (Table 2-6). Seed viability in all experimental treatments significantly decreased by 62 % between July/August 2007 and December 2007/January 2008 (Figure 5). Similar reductions in seed viability (80 % over 7 months) were reported in annual *Z. marina* beds in the Zandreek embayment in the Netherlands (Harrison, 1993). By definition, the seed-bank maintains seeds in a viable state under conditions favorable for germinating (Baker, 1989). In all seed-banks, once seeds have germinated, they are removed from the seed-bank; therefore, the number of viable seeds in the seed-bank decreases (Leck et al., 1989). The loss in the number of viable seeds in the Virginia and North Carolina experimental cores and ambient seed-bank reported here coincided with the period of maximum seed germination for *Z. marina* beds in these regions (Silberhorn et al., 1983; Thayer et al., 1984). Additional losses in the number of viable seeds were observed between the 6, 12, and 15 month sampling periods for both sites. While germination in *Z. marina* beds is still possible after December (Moore et al., 1993; Reusch, 2006), the additional seed loss reported here may also be due to damage to the seed coat, disease, or predation (Keddy and Patriquin, 1978; Harrison, 1993; Fishman and Orth, 1996).

Differences in the immediate sediment environment (including grain size, percent organic matter, sediment porewater nutrients, and redox potential) did not significantly affect the proportion of viable semi-annual and perennial *Z. marina* seeds remaining in the North Carolina experiment or in the perennial populations in the Virginia experiment (Table 2-6). These results were unexpected due to the results of controlled laboratory experiments which reported that sediment conditions such as redox potential or organic content significantly affect the success and timing of *Z. marina* seed germination (Churchill, 1992; Moore et al., 1993; Probert and Brenchly, 1999; Jarvis Chapter 3). The

lack of a sediment effect may have been an artifact of the sampling methodology. Moore et al. (1993) reported that seed germination occurred earlier in *Z. marina* seeds held under anoxic conditions than seeds under oxygenated conditions; however, after an extended period of time, seed germination did occur in the oxygenated treatments. By not sampling throughout the period of maximum germination, any sediment effects due to removal of viable seeds from the seed-bank via germination may have been missed. While sampling in 6 month intervals provided information on the type of seed-bank *Z. marina* beds produce (transient or persistent), research with finer temporal sampling schemes may be required to gain a more comprehensive understanding of sediment effects on semi-annual and perennial seed-bank viability.

Surprisingly, seed source did not have a significant effect on seed viability in either the North Carolina or Virginia experimental treatments (Table 2-6). These results were unexpected due to the reliance of annual and semi-annual beds on seeds for bed re-establishment on a yearly basis (Harper, 1977; Keddy and Patriquin, 1978; Phillips et al., 1983; Santamaría-Gallegos et al., 2000; Jarvis Chapter 1). While seeds from semi-annual beds were not significantly more viable than perennial seeds, there were significantly greater densities of seeds in the semi-annual compared to perennial site seed-banks (Table 2-3). Similar ambient seed-bank densities were reported for perennial (0 – 1,200 seeds m⁻²) and annual (1,300 – 30,000 seeds m⁻²) *Z. marina* populations throughout the species geographic range (Harrison, 1993; Harwell and Orth, 2002; Lee et al., 2007; Mortia et al., 2007). Greater densities in semi-annual compared to perennial seed-banks may be the result of increased seed production (up to seven times greater) in semi-annual compared to perennial beds (Keddy, 1987; Jarvis Chapter 1). Greater seed abundances

within the semi-annual *Z. marina* bed would increase the ability of the bed to re-establish on a yearly basis from seed (Inglis, 2000). Therefore by maintaining similar seed viabilities to perennial populations and producing significantly greater numbers of seeds, semi-annual *Z. marina* populations may have a greater regeneration potential from the seed-bank than perennial beds.

Viable seeds were only collected from Virginia seed-banks in October 2008. Low seed-bank densities in 2007 may be related to a large scale decline of *Z. marina* in the lower Chesapeake Bay in the summer of 2005 (Moore and Jarvis, 2008). Although cover and vegetative shoot density recovered to pre-decline levels at CB2 and to a lesser extent at CB1 by 2007 (Jarvis, Chapter 4), average reproductive shoot density was reduced to less than 1 % of total shoot density at both sites. Historically within this region, reproductive shoot densities range between 11 to 19 % of total shoot density (Silberhorn et al., 1983). Reduced flowering in 2007 would have limited seed-bank replenishment which may have resulted in non-viable seed-banks throughout the 2007 sampling period. Interestingly, seeds were collected from the CB2 sediment seed-bank in October 2008 and not in the July 2008 samples (Table 2-3). Although the collection of ambient seed-bank characterization samples was designed to represent the seed-bank during that sampling period, seed-banks are hard to quantify due to their inherent spatial heterogeneity (Harwell and Orth, 2002). In addition, seed-banks are not static environments and seeds are deposited and removed throughout the year (Leck et al., 1989). Seeds collected in the October 2008 sampling may have been introduced into the sediment seed-bank from rafting reproductive shoots after the July sampling. (Källström

et al., 2008). Regardless of the mechanism for which seeds were introduced into CB sediment seed-banks in 2007 and 2008 overall viable seed densities were extremely low.

The lack of long term viability in seed-banks could have a significant impact on the resilience of *Z. marina* populations to disturbance (Keddy and Patriquin, 1978; Plus et al., 2003; Greve et al., 2005). Perennial populations primarily rely on asexual reproduction for bed maintenance and expansion into nearby areas (Olsen, 1994; Olsen, 2004; Inglis, 2000). However, when the majority of above-ground biomass is removed from the bed due, for example, to anoxic conditions (Plus et al., 2003; Greve et al., 2005), high summer water temperatures (Moore and Jarvis, 2008), or by increased light attenuation from a large scale algae bloom (Lee et al., 2007) sexual reproduction in the form of seed germination from the seed-bank provides the recovery mechanism. *Z. marina* seedlings in biennial life history beds do not flower during their first growing season (Setchell, 1929; den Hartog, 1970; Silberhorn et al., 1983); therefore, the sediment seed-bank is not replenished for at least one year post decline and the resiliency provided by the seed-bank may be lost (Jarvis Chapter 4). Long term persistence of perennial *Z. marina* beds may be reduced by fewer viable seeds in the sediment seed-bank if there are consecutive years of disturbance.

Semi-annual beds may not be as susceptible to multiple consecutive years of disturbance events as perennial beds due to the ability of seedlings to flower and replenish sediment seed-banks on a yearly basis. However, semi-annual beds still produce transient seed-banks; therefore, the timing of disturbance events may be extremely important. *Z. marina* seed viability is reduced after the period of maximum germination and the seed-bank is not replenished until the following late spring/early

summer after seeds are released from flowering shoots (Silberhorn et al., 1983; Thayer et al., 1984). During this time period seedlings have germinated and seed-bank viability is at a low level. If a large scale disturbance, resulting in the reduction of above ground biomass, occurs before seedlings are able to flower, the sediment seed-bank is reduced and the recovery of the semi-annual bed may be limited. As large storms are predicted to increase in frequency and intensity and global water temperatures continue to rise (Najjar, 1999; Gibson and Najjar, 2000), *Z. marina* beds may be under increasingly stressful conditions and their resilience to disturbance may be limited by the transient nature of seed-banks.

Conclusions

While the results presented here indicate that both perennial and semi-annual *Z. marina* populations in North Carolina and Virginia produced transient viable sediment seed-banks the environmental and physiological factors that affect seed viability are still unclear. Research into the effect of time on both semi-annual and perennial *Z. marina* seed viability over finer time scales (< 6 months) is required to gain a better understanding of the effect of sediment composition on seed viability. In addition, future research directly comparing the regional difference in seed density and viability between *Z. marina* populations is required to fully understand sediment seed-bank dynamics within these valuable ecosystems.

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Table 2-1. A priori equation selection for both North Carolina and Virginia. (A) ambient seed-bank and (B) seed core viability.

Ambient Seed-bank Model		k	Experimental Core Model		k
A			B		
1. Total Seed-bank Density			$V_1 = \alpha + \text{time}$		2
$V_1 = \beta_0 + \beta_1 \text{time} + \sigma \epsilon_1$		3	$V_2 = \alpha + \text{time} + \text{sediment}$		3
$V_2 = \beta_0 + \beta_2 \text{site} + \sigma \epsilon_2$		3	$V_3 = \alpha + \text{time} + \text{site}$		3
$V_3 = \beta_0 + \beta_1 \text{time} + \beta_2 \text{site} + \sigma \epsilon_3$		4	$V_4 = \alpha + \text{time} + \text{source}$		3
		4	$V_5 = \alpha + \text{time} + \text{source} + \text{time} * \text{source}$		4
2. Proportion of Viable Seeds			$V_6 = \alpha + \text{time} + \text{sediment} + \text{site}$		4
$V_1 = \alpha + \beta_1 \text{time} + \sigma \epsilon_1$			$V_7 = \alpha + \text{time} + \text{sediment} + \text{source}$		4
$V_2 = \alpha + \beta_2 \text{site} + \sigma \epsilon_2$		2	$V_8 = \alpha + \text{time} + \text{site} + \text{source}$		4
$V_3 = \alpha + \beta_1 \text{time} + \beta_2 \text{site} + \sigma \epsilon_3$		2	$V_9 = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source}$		5
$V_4 = \alpha + \beta_1 \text{time} + \beta_2 \text{site} + \beta_3 \text{time} * \text{site} + \sigma \epsilon_4$		3	$V_{10} = \alpha + \text{time} + \text{site} + \text{source} + \text{time} * \text{site}$		5
		4	$V_{11} = \alpha + \text{time} + \text{site} + \text{source} + \text{time} * \text{source}$		5
			$V_{12} = \alpha + \text{time} + \text{site} + \text{source} + \text{time} * \text{site} + \text{time} * \text{source}$		6
			$V_{13} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time} * \text{sediment}$		5
			$V_{14} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time} * \text{source}$		5
			$V_{15} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time} * \text{sediment} + \text{time} * \text{source}$		6
			$V_{16} = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source} + \text{time} * \text{site} + \text{time} * \text{sediment} + \text{time} * \text{source}$		8
			$V_{17} = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source} + \text{time} * \text{site} + \text{time} * \text{sediment} + \text{time} * \text{source} + \text{site} * \text{sediment} + \text{site} * \text{source} + \text{sediment} * \text{source} + \text{time} * \text{site} * \text{sediment} + \text{time} * \text{site} * \text{source} + \text{site} * \text{sediment} * \text{source} + \text{time} * \text{site} * \text{sediment} * \text{source}$		15

Table 2-2. Average ambient and experimental sediment conditions for both (A) North Carolina and (B) Virginia sites. Values are averaged across depths and over time, and are presented as means \pm standard errors.

Core Site Sediment Type	Ambient		Experimental			
	NC/CB1	NC/CB2	NC/CB1		NC/CB2	
	Mud	Sand	Mud	Sand	Mud	Sand
A. North Carolina						
% Organic	3.27 \pm 0.16	1.19 \pm 0.07	2.70 \pm 0.19	2.27 \pm 0.34	2.93 \pm 0.67	1.31 \pm 0.10
Sand:Silt:Clay						
% Sand	71.18 \pm 1.46	88.35 \pm 1.23	81.00 \pm 1.94	90.13 \pm 2.52	78.31 \pm 0.29	91.38 \pm 3.70
% Silt	21.57 \pm 1.10	10.36 \pm 1.08	13.45 \pm 1.27	6.75 \pm 1.93	15.20 \pm 0.17	5.88 \pm 2.91
% Clay	7.25 \pm 0.52	1.30 \pm 0.18	5.55 \pm 1.76	3.11 \pm 0.65	6.49 \pm 0.30	2.74 \pm 0.79
Nutrients						
NH ₃ μ M	23.8 \pm 3.5	15.3 \pm 1.0	40.6 \pm 8.5	68.8 \pm 19.5	45.7 \pm 9.7	56.6 \pm 15.2
PO ₄ ⁻³ μ M	0.6 \pm 0.1	1.2 \pm 0.3	1.1 \pm 0.5	0.3 \pm 0.1	0.7 \pm 0.2	0.6 \pm 0.2
B. Virginia						
% Organic	2.71 \pm 0.18	0.90 \pm 0.03	2.14 \pm 0.15	0.76 \pm 0.11	1.76 \pm 0.21	0.82 \pm 0.11
Sand:Silt:Clay						
% Sand	61.00 \pm 4.49	87.93 \pm 1.35	65.35 \pm 1.74	88.67 \pm 4.66	81.44 \pm 2.83	94.10 \pm 0.72
% Silt	29.98 \pm 3.57	8.61 \pm 1.29	26.40 \pm 1.99	8.38 \pm 4.09	12.81 \pm 2.36	3.50 \pm 0.66
% Clay	9.02 \pm 1.17	3.46 \pm 0.78	8.25 \pm 0.81	2.96 \pm 0.61	5.74 \pm 0.82	2.40 \pm 0.20
Nutrients						
NH ₃ μ M	31.9 \pm 4.0	12.7 \pm 1.1	41.8 \pm 6.6	68.4 \pm 17.6	58.7 \pm 10.0	38.3 \pm 5.4
PO ₄ ⁻³ μ M	0.0 \pm 0.0	0.2 \pm 0.0	0.5 \pm 0.1	0.4 \pm 0.1	0.2 \pm 0.1	0.3 \pm 0.1

Table 2-3. Ambient sediment seed-bank total seed density, viable seed density, and percentage of seed viability in all North Carolina and Virginia sites. Values are mean \pm standard error.

Date	Site	Total m ⁻²	Viable m ⁻²	% Viable m ⁻²
North Carolina				
Jul-07	NC 1	342 \pm 138	26 \pm 26	10 \pm 10
	NC 2	316 \pm 105	79 \pm 35	33 \pm 21
Dec-07	NC 1	232 \pm 117	0 \pm 0	0 \pm 0
	NC 2	421 \pm 145	0 \pm 0	0 \pm 0
Jun-08	NC 1	147 \pm 42	0 \pm 0	0 \pm 0
	NC 2	906 \pm 246	358 \pm 184	18 \pm 9
Sep-08	NC 1	147 \pm 79	0 \pm 0	0 \pm 0
	NC 2	1243 \pm 147	147 \pm 63	10 \pm 4
Virginia				
Aug-07	CB 1	0 \pm 0	0 \pm 0	0 \pm 0
	CB 2	0 \pm 0	0 \pm 0	0 \pm 0
Jan-08	CB 1	0 \pm 0	0 \pm 0	0 \pm 0
	CB 2	0 \pm 0	0 \pm 0	0 \pm 0
Jul-08	CB 1	0 \pm 0	0 \pm 0	0 \pm 0
	CB 2	0 \pm 0	0 \pm 0	0 \pm 0
Oct-08	CB 1	0 \pm 0	0 \pm 0	0 \pm 0
	CB 2	53 \pm 18	11 \pm 11	10 \pm 10

Table 2-4. Top ranked models describing the viability of (A) total density of the ambient seed-bank and the proportion of viable seeds in seed-bank and (B) experimental seed cores for both North Carolina and Virginia experiments. Rankings were based on differences in QAICc values. (†) denotes model used in data analysis.

Model	k	Log likelihood	QAIC	QAICc	Δi
A. Ambient Seed-bank					
Total Density – North Carolina					
$V_3 = \beta_0 + \beta_1 \text{time} + \beta_2 \text{site} + \sigma \varepsilon_3^\dagger$	4	130.4	-254.7	-254.7	0.0
$V_1 = \beta_0 + \beta_2 \text{site} + \sigma \varepsilon_1^\dagger$	3	129.1	-254.3	-254.2	0.5
$V_2 = \beta_0 + \beta_1 \text{time} + \sigma \varepsilon_2$	3	122.4	-240.8	-240.8	13.9
Proportion of Viable Seeds – North Carolina					
$V_3 = \alpha + \beta_1 \text{time} + \beta_2 \text{site}^\dagger$	3	-59.5	124.9	124.9	0.0
$V_4 = \alpha + \beta_1 \text{time} + \beta_2 \text{site} + \beta_3 \text{time} * \text{site}$	4	-58.6	125.3	125.3	0.4
$V_2 = \alpha + \beta_1 \text{time}$	2	-63.1	130.3	130.3	5.4
$V_1 = \alpha + \beta_2 \text{site}$	2	-69.9	143.8	143.8	18.9
B. Experimental Seed Core					
North Carolina					
$V_{15} = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source} + \text{time} * \text{site} + \text{time} * \text{sediment} + \text{time} * \text{source}^\dagger$	8	-941.3	1898.6	1898.7	0.0
$V_{14} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time} * \text{sediment} + \text{time} * \text{source}$	6	-948.7	1909.4	1909.4	10.7
$V_{12} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time} * \text{sediment}$	5	-953.1	1916.2	1916.2	17.5
$V_{11} = \alpha + \text{time} + \text{site} + \text{source} + \text{time} * \text{site} + \text{time} * \text{source}$	6	-953.7	1919.4	1919.4	20.7
Virginia					
$V_5 = \alpha + \text{time} + \text{source} + \text{time} * \text{source}^\dagger$	4	-965.5	1939.1	1939.1	0.0
$V_{14} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time} * \text{source}$	5	-965.4	1940.7	1940.8	1.7
$V_{11} = \alpha + \text{time} + \text{site} + \text{source} + \text{time} * \text{source}$	5	-965.5	1941.0	1941.0	1.9
$V_{10} = \alpha + \text{time} + \text{site} + \text{source} + \text{time} * \text{site}$	5	-977.9	1965.9	1965.9	6.8

Table 2-5. North Carolina ambient seed-bank (A) density and (B) viability models and odds ratios. (A) Density values calculated with negative binomial regression model. (B) Viability values calculated with logistic regression model. All significant results are denoted with an (*).

Parameter	DF	Est	SE	X ²	p	odds ratio	Wald 95 % CL	
							Low	High
A. Total Density								
$V_3 = \beta_0 + \beta_1\text{time} + \beta_2\text{site} + \sigma\epsilon_3$								
Intercept	1	0.63	0.33	3.62	0.057	1.88	0.98	3.60
Time: July 07 (0 mo)	0	--	--	--	--	--	--	--
Time: Dec 07 (6 mo)	1	-0.21	0.41	0.26	0.608	0.81	0.37	1.80
Time: Jun 08 (12 mo)	1	0.12	0.40	0.10	0.753	1.13	0.52	2.47
Time: Sept 08 (15 mo)	1	0.36	0.39	0.85	0.356	1.44	0.67	3.10
Site	1	1.22	0.26	21.55	<0.001*	3.39	2.02	5.81
Dispersion	1	0.29	0.15	--	--	--	--	--
$V_1 = \beta_0 + \beta_2\text{site} + \sigma\epsilon_1$								
Intercept	1	0.69	0.22	10.35	0.001*	2.00	1.31	3.05
Site	1	1.29	0.27	22.17	<0.001*	3.64	2.13	6.23
Dispersion	1	0.38	0.16		--	--	--	--
B. Proportion Viable								
Intercept	1	-3.24	1.09	8.86	0.003*	0.04	0.00	0.33
Time 0 mo	0	--	--	--	--	--	--	--
Time 6 mo	1	-24.94	--	--	--	--	--	--
Time 12 mo	1	0.48	0.75	0.41	0.520	1.62	0.37	7.10
Time 15 mo	1	-1.07	0.80	1.78	0.182	0.34	0.07	1.65
Site	1	2.29	1.09	4.42	0.036*	9.89	1.17	83.68

Table 2-6. Logistic regression model and calculated odds ratios for (A) North Carolina and (B) Virginia experimental seed viability cores. All significant results are denoted with an (*).

							Wald 95 % CL	
Parameter	DF	Est	SE	X ²	p	odds ratio	Low	High
North Carolina								
Intercept	1	1.09	0.14	64.33	<0.001*	2.97	2.28	3.88
Time 0 mo	0	--	--		--	--	--	--
Time 6 mo	1	-5.07	0.47	117.94	<0.001*	0.01	0.00	0.02
Time 12 mo	1	-5.92	1.08	30.24	<0.001*	0.00	0.00	0.02
Time 15 mo	1	-5.47	0.69	62.84	<0.001*	0.00	0.00	0.02
Site	1	0.29	0.14	4.29	0.038*	1.33	1.02	1.75
Sediment	1	0.00	0.14	0.00	1.000	1.00	0.76	1.31
Source	1	0.02	0.14	0.02	0.890	1.02	0.78	1.34
Site*Time 0 mo	0	--	--		--	--	--	--
Site*Time 6 mo	1	-0.41	0.34	1.42	0.233	0.66	0.34	1.30
Site*Time 12 mo	1	1.72	1.05	2.69	0.101	5.56	0.71	43.25
Site*Time 15 mo	1	0.83	0.66	1.58	0.208	2.30	0.63	8.42
Sed*Time 0 mo	0	--	--		--	--	--	--
Sed*Time 6 mo	1	1.82	0.47	15.32	<0.001*	6.19	2.49	15.44
Sed*Time 12 mo	1	0.30	0.54	0.31	0.579	1.35	0.47	3.89
Sed*Time 15 mo	1	0.28	0.53	0.27	0.602	1.32	0.47	3.73
Source*Time 0 mo	0	--	--		--	--	--	--
Source*Time 6 mo	1	-0.96	0.38	6.22	0.013*	0.38	0.18	0.81
Source*Time 12 mo	1	-0.73	0.54	1.84	0.175	0.48	0.17	1.39
Source*Time 15 mo	1	0.30	0.53	0.31	0.575	1.34	0.48	3.79
Virginia								
Intercept	1	-0.36	0.19	3.85	0.05	0.69	0.48	1
Time 0 mo	0	--	--	--	--	--	--	--
Time 6 mo	1	0.85	0.72	1.3	0.254	2.33	0.54	10
Time 12 mo	1	-4.46	1.14	15.32	<0.001*	0.01	0.00	0.11
Time 15 mo	1	16.1	0.71	509.08	<0.001*	--	--	--
Source	1	0	0.12	0	1.000	1.000	1.26	1.26
Source*Time 0 mo	0	--	--	--	--	--	--	--
Source*Time 6 mo	1	-2.55	0.63	16.47	<0.001*	0.08	0.02	0.27
Source*Time 12 mo	1	0.09	0.72	0.01	0.903	1.09	0.27	4.48
Source*Time 15 mo	1	-21.05	--	--	--	--	--	--

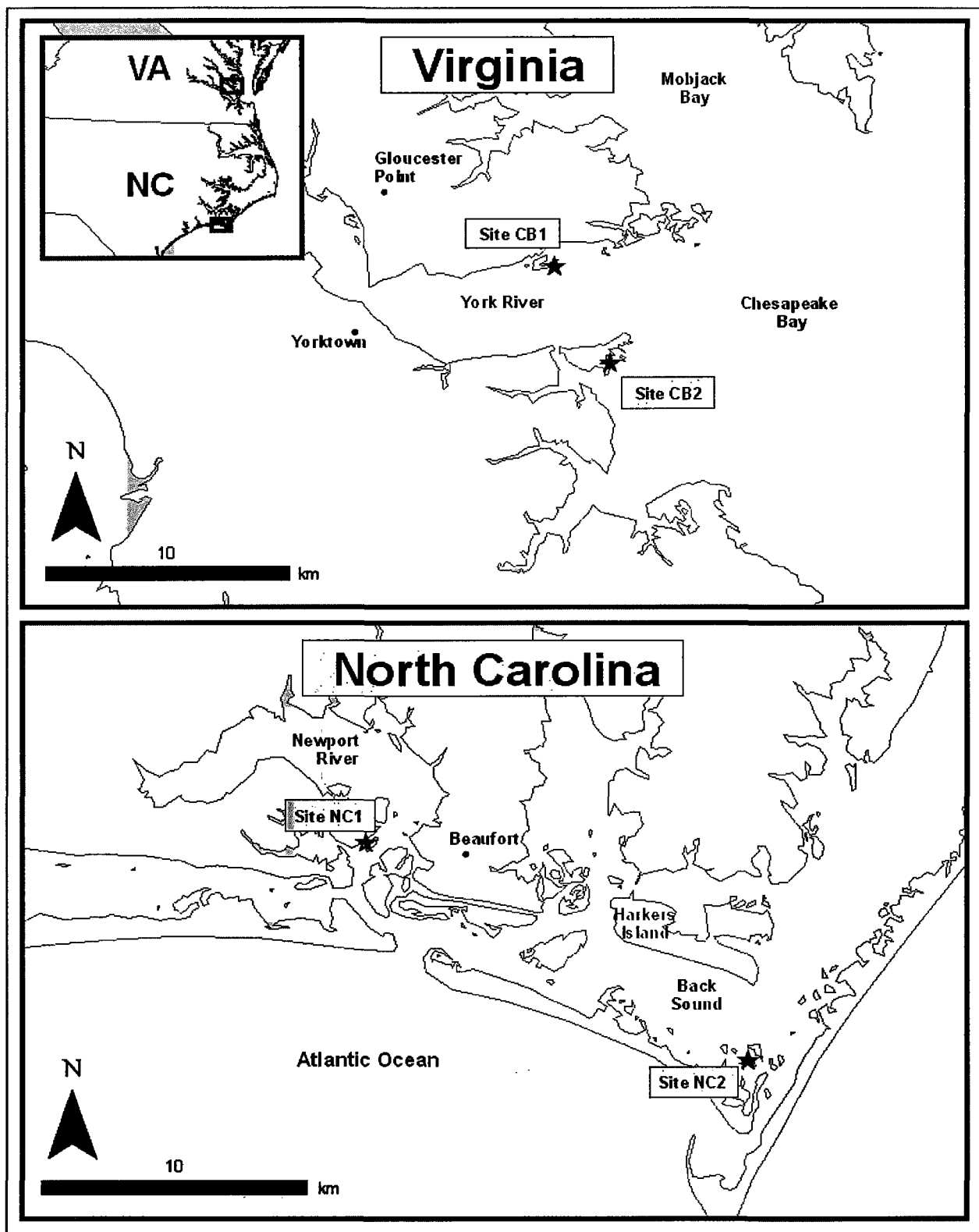


Figure 2-1. Site locations for both the Virginia (CB1 and CB2) and North Carolina (NC1 and NC2) experiments. Site locations are denoted with a star.

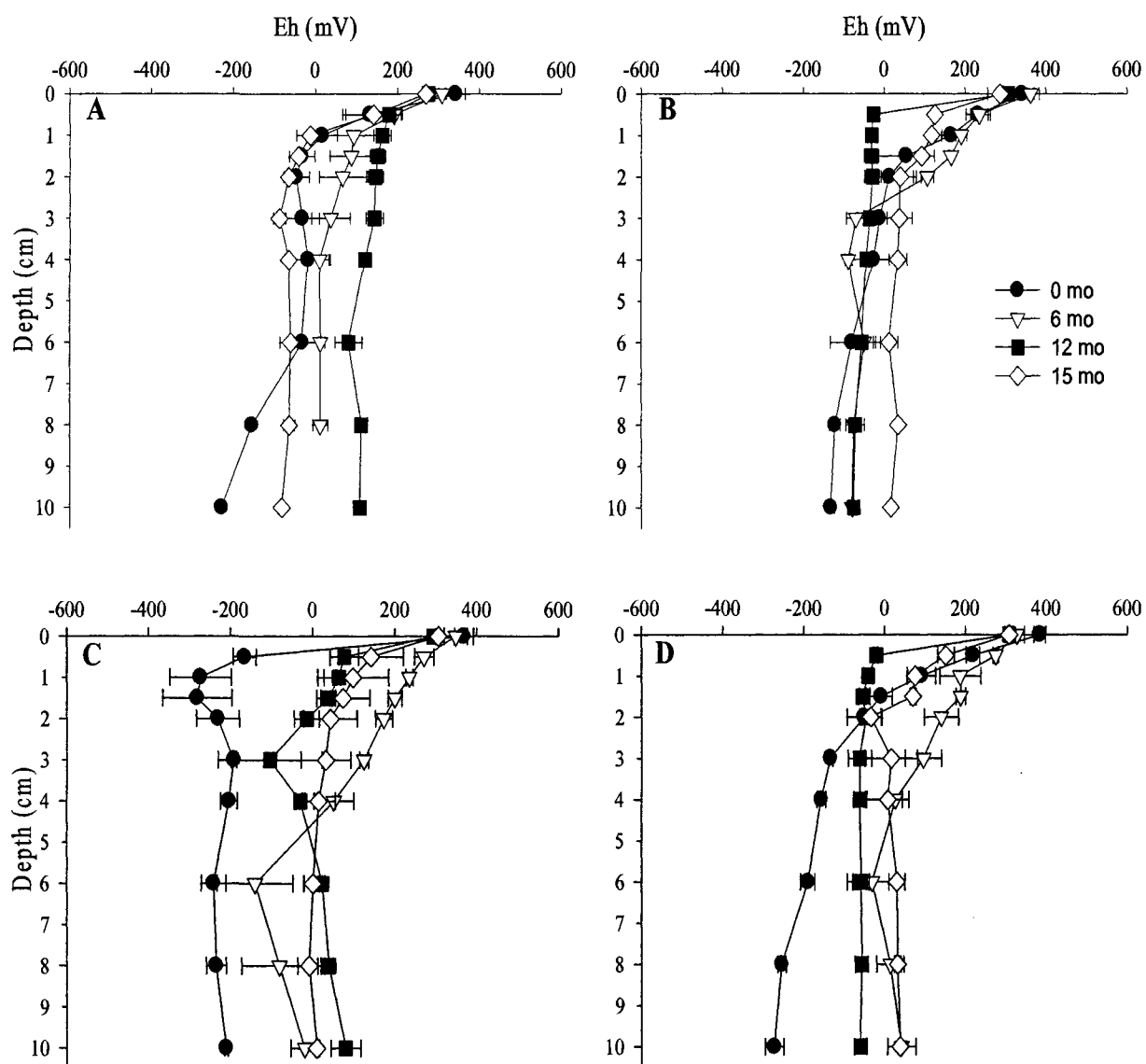


Figure 2-2. Mean sediment redox profiles for all sampling sites (A) NC1; (B) NC2; (C) CB1 (D) CB2 across all sampling dates. Values are mean \pm standard error.

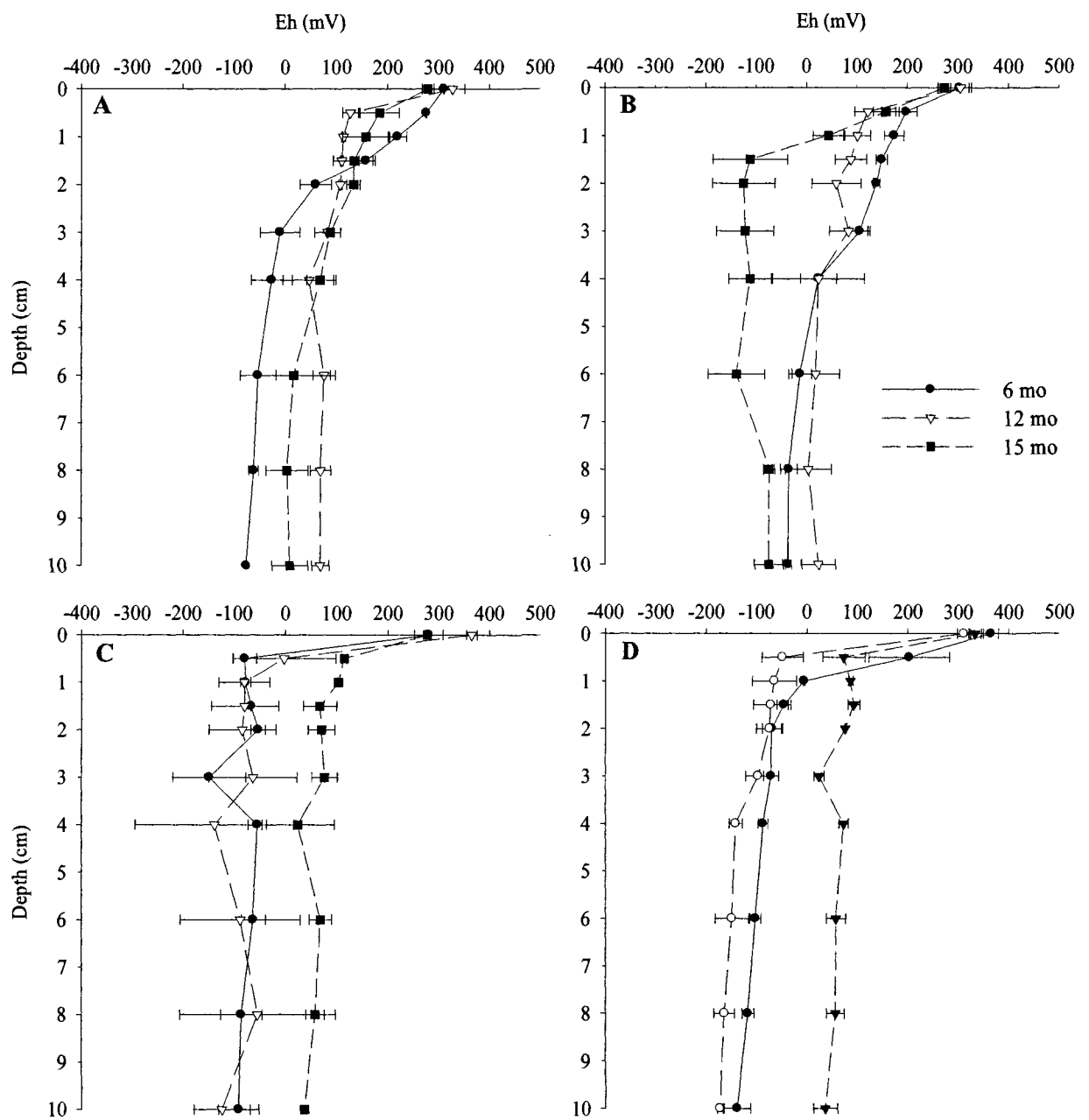


Figure 2-3. Mean sediment redox profiles for each sediment treatment averaged across both North Carolina sites. (A) sand sediment perennial seeds; (B) sand sediment annual seeds (C) mud sediment perennial seeds, (D) mud sediment semi-annual seeds. Values are mean \pm standard error.

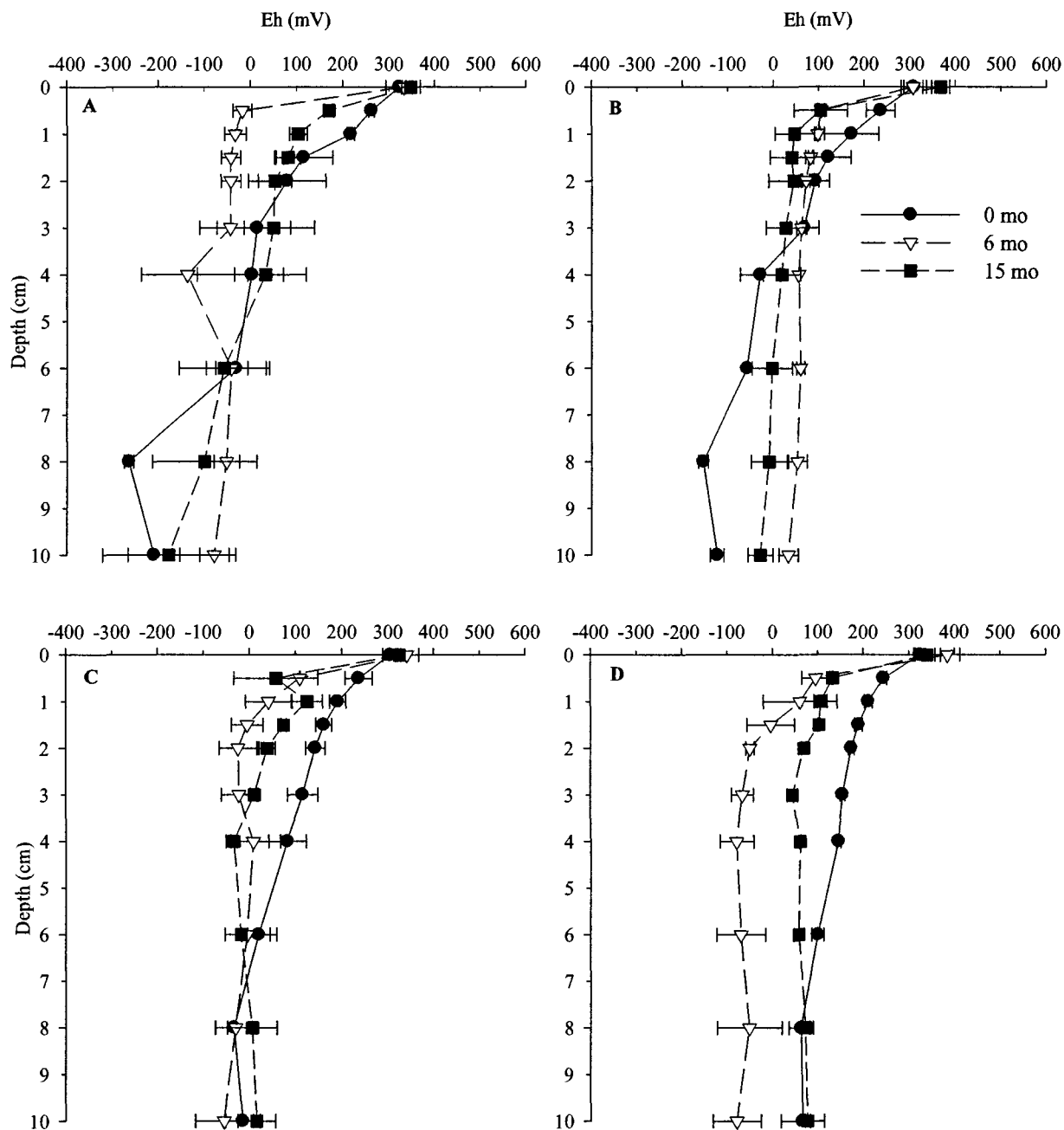


Figure 2-4. Mean redox values for experimental sediment cores averaged across both Virginia sites. (A) mud sediment, perennial-mud seeds (B) mud sediment, perennial-sand seeds, (C) sand sediment, perennial-mud seeds, (D) sand sediment, perennial-sand seeds. Values are mean \pm standard error.

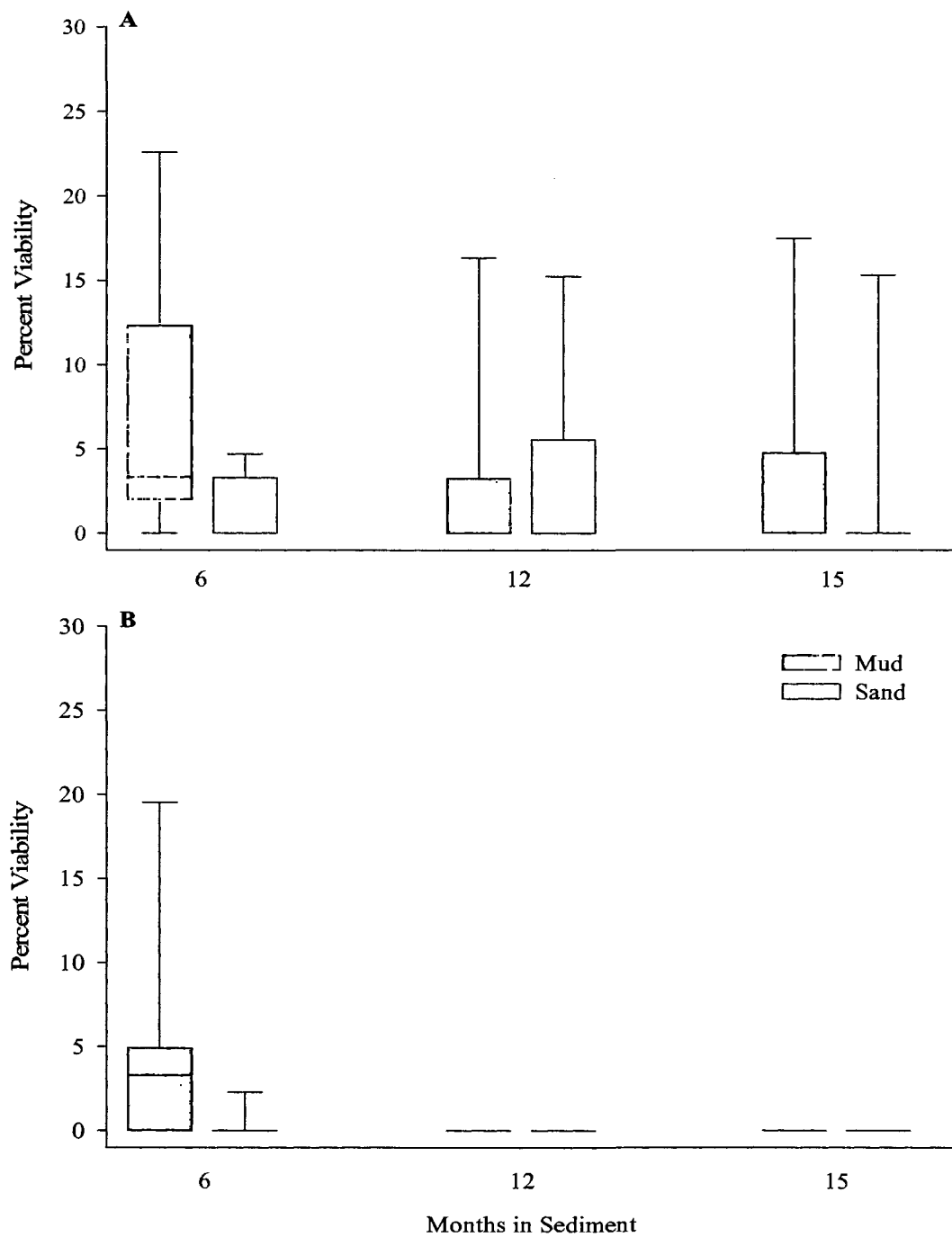


Figure 2-5. Viability of remaining seeds in both (A) North Carolina and (B) Virginia experimental seed cores. Dark gray bars represent seeds in mud treatments and white bars represent seeds in sand treatments.

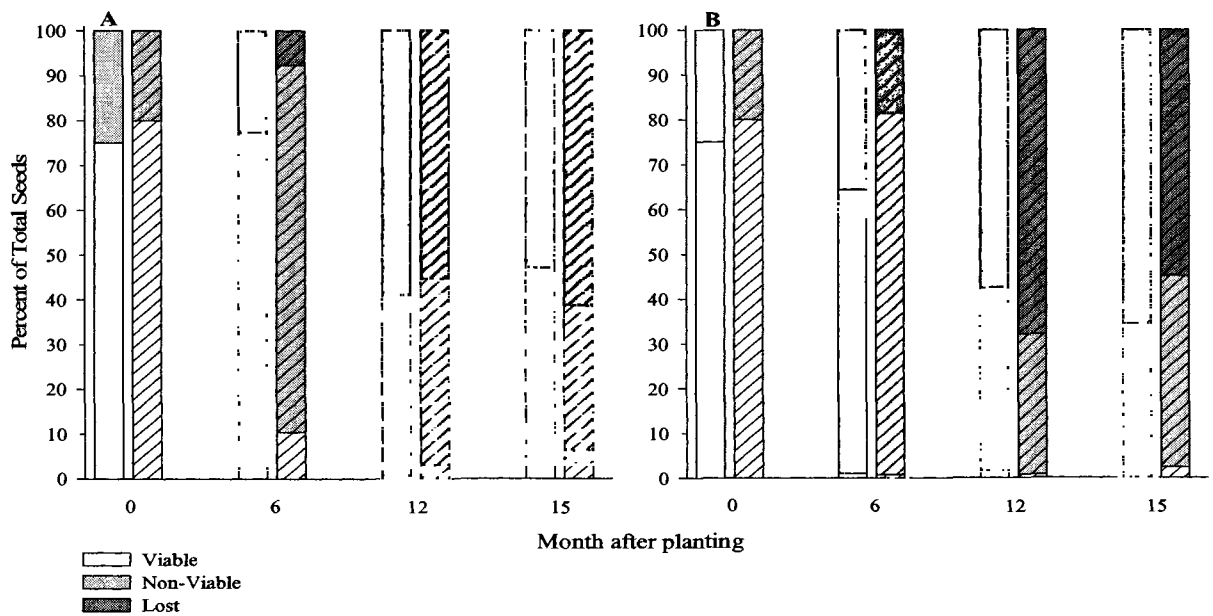


Figure 2-6. Mean percent of total seeds removed from each experimental sediment core in North Carolina sites. Solid bars = semi-annual seed. Bars with hash marks = perennial seeds. Sediment types are A =mud, B = sand.

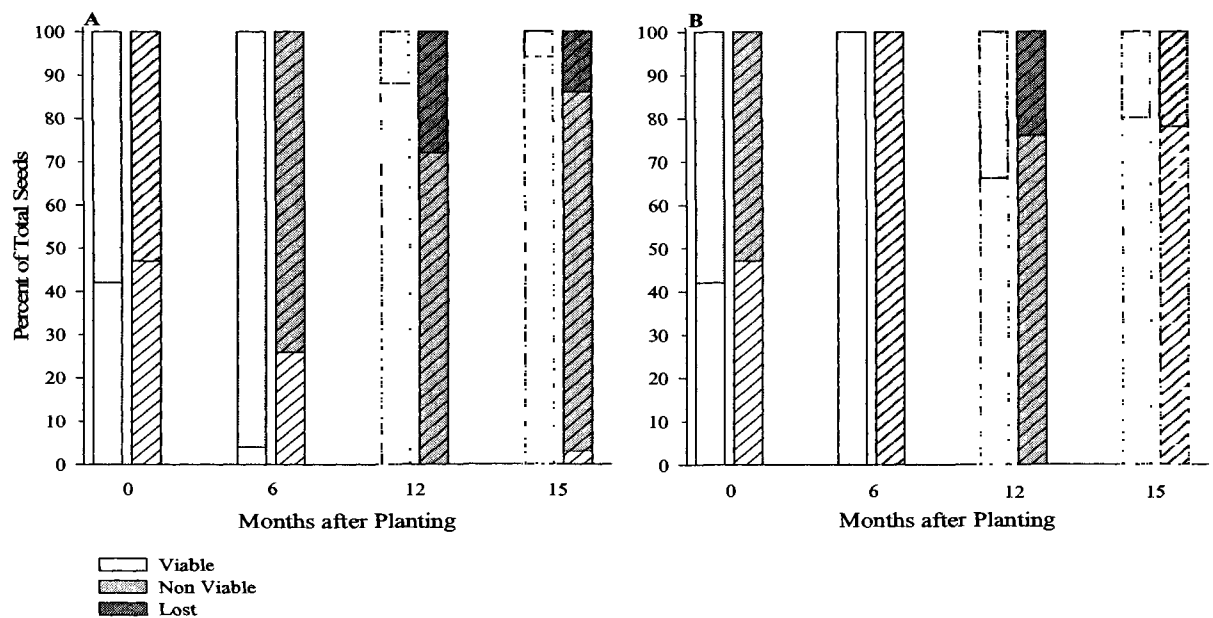


Figure 2-7. Mean percent of total seeds removed from each experimental sediment core in Virginia sites. Solid bars = semi-annual seed. Bars with hash marks = perennial seeds. Sediment types are A =mud, B = sand.

CHAPTER 3 : SEEDLING EMERGENCE AND SEED VIABILITY OF SEMI-
ANNUAL AND PERENNIAL ZOSTERA MARINA L. SEEDS: THE EFFECTS OF
SEED SOURCE, SEDIMENT TYPE, AND BURIAL DEPTH

Abstract

Maximum seedling emergence, time to emergence, remaining seed viability, and initial seedling biomass were compared between semi-annual and perennial *Zostera marina* seed populations in sandy (< 1 % organic content) and muddy (> 3 % organic content) sediments and at shallow (1 cm) and deep (5 cm) depths. Perennial seeds collected from the York River, Virginia and from Back Sound, North Carolina had significantly greater maximum percent germination, shorter time to germination, and greater seedling biomass compared to semi-annual seeds collected from the Newport River, North Carolina. For both semi-annual and perennial seeds, emergence was greatest in shallow muddy sediments although mean time to germination was not significantly different. Viability of the remaining seeds was not significantly affected by burial depth or sediment type; however viability for all seed sources was < 10%. The percentage of non-viable but pre-emergent seeds (embryo split from the seed coat, but did not emerge from the sediment surface) was significantly greater in deep treatments, especially for perennial seeds in the sandy treatment. Both perennial and semi-annual initial seedling biomass was greater in muddy compared to sandy sediments and in shallow compared to deep depths. The results reported here suggest differing strategies between western mid-Atlantic semi-annual and perennial *Z. marina* populations for successful seedling establishment. Semi-annual populations use a “bet hedging” strategy producing smaller, less fit seeds that are able to disperse to more “safe sites” and escape local stressful conditions while perennial populations produce fewer larger seeds that have higher emergence rates increasing their competitive fitness within the more stable perennial bed. While there was no significant regional difference in seedling emergence between perennial *Z. marina* sources, sediment

conditions did significantly affect seedling emergence, highlighting the role of the surrounding sediment rather than the location of the source population on successful sexual reproduction within mid-Atlantic perennial *Z. marina* beds. Reduced seedling emergence of both semi-annual and perennial *Z. marina* seeds at burial depths > 1 cm may represent a possible bottleneck in successful *Z. marina* sexual reproduction. Implications of reduced *Z. marina* seed viability due to burial depth or sediment conditions may affect the resiliency to and recovery from disturbance for both perennial and semi-annual *Z. marina* beds.

Keywords: *Zostera marina*, semi-annual, seeds, sediment, viability

Introduction

Zostera marina, a dominant seagrass species in the temperate climates of the Northern Hemisphere, is found in perennial (den Hartog, 1970; Tomlinson, 1974), annual (den Hartog, 1970; Short and Moore, 2006), and semi-annual forms (Jarvis Chapter 1). Perennial *Z. marina*, primarily reproduces asexually (Setchell, 1929; Tutin, 1942; Thayer et al., 1984) although sexual reproduction is important for dispersal to new habitats (Harwell and Orth, 2002a; Källström et al., 2008) and recovery from large scale declines (Plus et al., 2003, Greve et al., 2005; Lee et al., 2007). Annual expressions of *Z. marina* rely completely on the production of seeds for establishment and are found most frequently in areas where environmental disturbance is historically greatest (Keddy and Patriquin, 1978; Phillips et al., 1983a; van Lent and Verschuure, 1994). Semi-annual forms of *Z. marina* rely on seeds for re-establishment on an annual basis, however they reproduce both sexually and asexually (Jarvis, Chapter 1).

For perennial *Z. marina* the production of flowering shoots varies between 10 – 19 % of total shoot density (Silberhorn et al., 1983; Harwell and Orth, 2002b), but may increase in response to disturbance (Phillips et al., 1983a; van Lent and Verschuure, 1994; Guidetti, 2000). Under the extreme conditions found at the limits of *Z. marina* distribution a few populations have become completely reliant on sexual reproduction (Phillips et al., 1983a; Meling-Lopez and Ibarra-Obando, 1999). As with other annual plant species, survival as seeds in a sediment seed-bank during stressful times of the year allows annual *Z. marina* populations to inhabit areas where severe environmental conditions such as high temperatures (Meling-Lopez and Ibarra-Obando, 1999; Santamaría-Gallegos, 2000) or ice scour (Keddy, and Patriquin, 1978; Phillips et al.,

1983a; Robertson and Mann, 1984) are lethal to perennial populations. More recently there has been a documented shift from perennial to an semi-annual form of *Z. marina* in a well studied *Z. marina* bed located near the southern limit of the species distribution along the western Atlantic in the Newport River, North Carolina (Kenworthy unpublished data.; Jarvis, Chapter 1). While it is hypothesized that the shift of this bed from perennial to a semi-annual form may be in response to increasing water temperatures or other environmental stressors the actual cause for the shift in reproductive strategy is unknown.

In addition to the shift from perennial to semi-annual reproductive strategy in one site in the southern limit of *Z. marina* along the western Atlantic, large scale population changes in the form of sudden and severe declines in *Z. marina* populations have also been documented in the Chesapeake Bay (Moore and Jarvis, 2008). As populations continue to decline, sexual reproduction may serve a vital role in the maintenance and recovery of *Z. marina* populations (Plus et al., 2003; Greve et al., 2005; Lee et al., 2007).. Despite the documented role of seeds in recovery of perennial *Z. marina* beds following large scale declines and the importance of seeds for maintaining annual and semi-annual populations, little is known concerning environmental and physiological factors that limit sexual reproduction in *Z. marina*.

Harper (1977) described seed germination as a potential limiting stage in angiosperm sexual reproduction. This was primarily attributed to the surrounding microenvironment which may lack the required signals to break seed dormancy and enhance germination (Baskin and Baskin, 1998; Woodin et al. 1998; Jurado and Flores, 2005). For *Z. marina* the primary environmental germination cues which perennial seeds have been most responsive to are changes in temperature (10-16 °C; Setchell, 1929;

Taylor, 1957; Lamounette, 1977), dissolved oxygen (anoxic conditions; Churchill et al., 1992; Moore et al., 1993; Probert and Brenchly, 1999), and sediment organic content (>1 %; Short, 1987; van Katwijk et al., 1997; van Katwijk and Wijgergangs, 2004). Due to the prevalence of perennial *Z. marina*, little comparison has been made between annual, semi-annual, and perennial populations to examine germination responses between semi-annual and perennial *Z. marina* seeds or the response of these two forms to the surrounding sediment microenvironment.

In the present study we quantified the effects of semi-annual and perennial seed sources, sandy (< 1 % organic content) and muddy (> 3 % organic content) sediments, and shallow (1 cm) and deep (5 cm) burial depths on time to emergence and maximum emergence of *Z. marina* seeds collected from the southern limit of the species western Atlantic populations. I hypothesized that maximum germination and shorter time to germination would occur in (1) semi-annual seeds compared to perennial seeds across all treatments; (2) seeds planted in muddy sediments compared to sandy sediments; (3) seeds planted in shallower depths compared to deep sediments.

Methods

Seed Collection and Viability

Reproductive shoots with mature flowers were collected from a semi-annual *Z. marina* bed at Phillips Island (NC1, 34° 43' N, 76° 41' W) in the Newport River, North Carolina and from a perennial bed at Morgans Island (NC2, 34° 66' N, 76° 52' W) in Back Sound, North Carolina. Reproductive shoots were also collected from two

perennial beds in the lower York River, a tributary to the Chesapeake Bay, at Allens Island (CB1, 37° 15' N; 76° 25' W) and Goodwin Island (CB2, 37° 13' N; 76° 23' W). Reproductive shoots were collected from *Z. marina* beds during the period of maximum seed release; early to late May in North Carolina (Phillips et al., 1983b; Thayer et al., 1984) and late May and early June in Chesapeake Bay (Silberhorn et al., 1983). Reproductive shoots were kept in separate aerated flow-through tanks (2.4 m x 1.2 m x 1.0 m) until seeds dehisced from the reproductive shoots (Orth et al., 2007). The seeds were then collected from the bottom of the tank and kept in separate containers in aerated recirculating tanks (2.4 m x 1.2 m x 1.0 m) at 20 °C until placement in experimental cores.

A sub-sample of 100 dark mature seeds from each seed source was tested for viability using tetrazolium chloride (Lakon, 1949; AOSA 1981; Sawma and Mohler, 2002). Seed embryos were removed from their seed coats and soaked in a 1 % tetrazolium chloride solution for 24 hours before examination with a dissecting scope at 10 x magnification (Conacher et al., 1994). Seeds with a red to brown stained cotyledon were considered viable (Taylor, 1957).

Sediment Collection and Characterization

Sediment was collected from established *Z. marina* beds at CB1 (high organic, > 3 %) and CB2 (low organic, < 1 %) and stored at 7 – 9 °C until processing. Prior to analysis all sediment was sieved (0.5 mm mesh) to remove extraneous seeds then homogenized. Five sediment plus (11.4 cm diameter by 2 cm depth) were collected from each sediment mixture and quartered. The first quarter was sieved (63 µm sieve),

washing silt and clay fractions into a graduated cylinder. After 24 hours, pipette analysis was performed to determine the clay (8 phi) and silt (4 phi) fractions of the sample (modification of Plumb, 1981). Dry weights of the aliquots were then compared and percent sand silt and clay fractions were determined. All sediment was classified based on sand/silt/clay ratios (Shepard, 1954).

Percent organic matter in the sediment was determined by drying two quarters of the sediment sub-sample at 60 °C for 5 days or until a constant dry weight was reached. The samples were cooled in a desiccator and 10 g of sediment was weighed and combusted at 500 °C for five hours. Each sample was weighed again and percent organic matter calculated (Erftemeijer and Koch, 2001). Sediment exchangeable nutrients were extracted from the fourth sample quarter in 2 M KCl, shaken for 1 hour, centrifuged at 4000 rpm for 6 minutes, filtered (Gelman Supor, 0.45 µm), and frozen until analysis. Dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphate (DIP) were determined using a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002).

Seed Source, Sediment Type, and Burial Depth Experiment

To quantify the interactive effects of seed source, sediment type, and burial depth, 25 seeds from each seed source (3 perennial beds, 1 semi-annual bed) were buried in sand (< 1% organic content) and mud (> 3% organic content) sediment treatments at depths of 1 and 5 cm in PVC cores (6.0 cm in diameter, 12.0 cm). Each core was replicated three times for all treatments and placed in a large recirculating tank (1.2 m x 2.4 m x 1.0 m) in a randomized complete block design. After initial testing indicated a maximum period of

1 to 2 days between seed germination (seed coat split and radicle emerged) and the emergence of the cotyledon from the sediment surface (Jarvis unpublished data), seedling emergence was selected as an accurate measurement of successful germination and potential seedling establishment. Seedling emergence rather than germination was quantified because seedling emergence represented all seeds which germinated and emerged from the sediment surface minus those which died before the seedling could emerge from the sediment surface (Harper, 1977). Seedling emergence was quantified daily (Baskin and Baskin, 1998).

Water in each tank was maintained between 10-15 °C with an aquarium chiller. Water temperature (°C), salinity (PSS), dissolved oxygen (mg l^{-1}), and pH were measured daily with a Yellow Spring Instruments, Inc. (YSI, Inc., Yellow Spring, Ohio) model 650 sonde for 61 days. In addition three water samples were collected at day 1 and day 61, filtered (Gelman Supor, 0.45 μm), and frozen until analyzed for DIN and DIP with a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002). Water samples were also filtered and analyzed for chlorophyll *a* (Strickland and Parson, 1972) and total suspended solids (TSS). TSS was quantified from a well-mixed sample of known volume. The sample was filtered through a GF/F filter. The residue retained on the filter was dried to constant weight at 103 – 105 °C and reported as $\text{mg total suspended solids l}^{-1}$. Ambient incident irradiance was measured with a LI-COR, Inc. terrestrial sensor (LI-190SA).

Sediment samples from each treatment were collected a second time at day 61 and analyzed for organic content, sediment exchangeable nutrients, and grain size as described previously. In addition, vertical redox (Eh) profiles of all sediment types were

measured at day 61 with a 21 cm platinum electrode. The probe was inserted into the top of the core and redox was measured at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 cm. Final readings were corrected for temperature relative to the reference electrode (Hinchey and Schaffner, 2005).

After the experiment all cores were removed from the tank, the sediment was sieved (0.50 mm mesh), and all remaining seeds were counted and stored overnight at 4 °C. Viability of the remaining seeds was tested using tetrazolium chloride (Lakon, 1949; AOSA 1970; Sawma and Mohler, 2002). Non-viable embryos were divided into pre-emergent and intact seeds. Embryos which split the seed coat but did not emerge from the sediment surface were defined as pre-emergence seeds. Embryos from whole seeds that were completely contained within the seed coat but did not retain viability over the experimental period were defined as non-viable.

Germinated seedlings were removed from all experimental cores. Biomass samples were divided into flowering shoots or vegetative shoots. Vegetative shoots were counted then separated from the rhizome directly below the leaf sheath into above-ground and below-ground biomass samples. All biomass samples were dried at 60 °C until a constant dry weight to the nearest 0.01 g was reached.

Data Analyses

Prior to analysis data were transformed when necessary, normality was confirmed, and homogeneity of variance was verified with Cochran's test (Zar, 1999). Differences in sediment organic content and sediment exchangeable nutrients between sediment treatments were analyzed with separate one-way Analysis of Variance

(ANOVA; The SAS System for Windows, SAS Institute Inc.). A three-way ANOVA was used to compare the effects of source, sediment type, burial depth, and the interactions of these factors on redox measurements. All *post hoc* analyses of the data were performed with Tukey's HSD test (Tukey's Test; The SAS System for Windows, SAS Institute Inc.).

Maximum emergence data were analyzed using logistic regression with seed source, sediment type, and burial depth as factors (PROC GENMOD; SAS Institute Inc.; Scott et al., 1984). Logistic regression was used based on the assumption that germination of one seed did not significantly affect germination of other seeds (Orth et al, 2007). Therefore, each seed was considered an independent Bernoulli trial and analyzed separately (perennial $n = 75$; semi-annual $n = 25$). Initially the model describing maximum germination containing both seed sources failed to converge due to strong differences between source populations. Therefore, perennial and semi-annual seed responses were analyzed separately. Confidence intervals for both parameter estimates and odds ratios were calculated using the Wald Chi Square statistic (SAS; SAS Institute Inc.).

Survival analyses using LIFETEST and LIFEREG procedures were selected to quantify mean time to emergence and treatment effects on time to emergence (SAS; SAS Institute Inc). Survival analyses were selected due to the potential for a large amount of right-censored data characteristic of germination experiments (Scott et al., 1984). Seed data were censored if seedling emergence did not occur and non-emergent seeds were flagged prior to analysis. As with maximum seedling emergence, time to seedling emergence analysis of perennial and semi-annual seed populations was done separately.

Interaction terms were not included in the analysis of semi-annual and perennial seeds after the difference in log-likelihood functions between the analyses with and without interaction terms were non-significant.

Negative binomial regression was used to analyze the effects of sediment type, burial depth, and seed source on remaining semi-annual and perennial viable, non-viable, and pre-emergent seeds (GENMOD; The SAS System for Windows, SAS Institute Inc.). Over dispersion was accounted for using a Pearson chi-square correction factor. Model selection for both semi-annual and perennial seed sources was based on log-likelihood estimates.

Biomass data were transformed using 4th root transformation techniques (Zar, 1999) and analyzed with Analysis of Variance (ANOVA; The SAS System for Windows, SAS Institute Inc.). The three-way ANOVA compared the effects of seed source, sediment type, burial depth, and the interactions of these factors on total seedling biomass. All *post hoc* analyses of the data were performed with Tukey's test (Tukey's Test; The SAS System for Windows, SAS Institute Inc.).

Results

Water Quality Conditions

Water quality conditions remained consistent through the 61 day sampling period. Average water temperature was 14.2 ± 0.8 °C, salinity was 28.7 ± 0.5 , dissolved oxygen was 9.84 ± 0.54 mg l⁻¹, and pH was 7.80 ± 0.14 . Water column NH₄⁺ levels on average were 1.77 ± 0.20 μM and PO₄⁻³ levels were 1.88 ± 0.29 μM. Water column conditions were comparable to conditions in both the Chesapeake Bay and in the Newport River and

Back Sound during periods of *Z. marina* germination (Chesapeake Bay Program, 2008; Jarvis Chapter 1).

Sediment Characteristics

Sediment organic content was significantly different between the two sediment sources ($p = 0.005$). The mean organic content for the sand treatment was 0.4 % and 3.1 – 3.3 % for the mud treatment (Table 3-1). The sand treatment was comprised primarily of sand, 96.5 – 96.7 %, and was classified as sandy substrate. The mud treatment was sandy silt and contained 42.0 % sand, 44.2 % silt, and 14.5 % clay. Sediment exchangeable NH_4^+ was significantly greater in the deep compared to shallow treatments ($p = 0.005$) and in the muddy compared to sandy sediments ($p = 0.015$). Sediment PO_4^{-3} levels were generally low ($< 1.0 \mu\text{M}$) however sand treatments had significantly greater PO_4^{-3} concentrations than muddy sediments ($p = 0.031$). PO_4^{-3} concentrations did not differ significantly with depth ($p = 0.532$). There was no interaction between depth and sediment on sediment nutrient concentrations in either NH_4^+ ($p = 0.226$) or PO_4^{-3} ($p = 0.966$).

Overall, redox levels were high in both sand and mud treatments (Figure 3-1). Sand treatments did not reach anoxic levels ($\text{Eh} < 0$) until depths greater than 8 cm, whereas mud treatments only experienced anoxia at 6 cm and were above anoxic levels at all other depths. Redox levels were significantly different between sediment types ($p = 0.049$) and depth ($p < 0.001$) and there was no interaction between sediment type and depth ($p = 0.952$). Tukey's *post hoc* test results indicate that redox levels at 0.0 and 0.5 cm were significantly different than all depths greater than 1.0 cm, but no other

depths were significantly different. Therefore after an initial decrease in redox levels over the first 1.0 cm, redox levels remained consistent with depth over both sediment types.

Maximum Seedling Emergence

Across all treatments perennial seedlings were found to be 11.3 times more likely to emerge from the sediment surface compared to semi-annual seeds (Table 3-2, Figure 3-2). These results were not related to differences in seed source viability as initial seed viability was similar across seed sources and ranged from 60 to 80 %. The greater maximum emergence of perennial seedlings was consistent between sediment types and over burial depths (Table 3-2, Figure 3-2). Within the Chesapeake Bay perennial populations (CB1 $p = 0.031$; CB2 $p = 0.002$) maximum seedling emergence was significantly different than North Carolina perennial seeds (Table 3-2). Chesapeake Bay seeds collected from the high organic sediment site (CB1) were 2.2 times more likely to emerge compared to North Carolina seeds collected from NC2, a site with low organic sediments (Table 3-2), whereas Chesapeake Bay seeds collected from the low organic sediment site (CB2) were only 0.8 times as likely to emerge compared to North Carolina seeds collected from low organic sediments (Table 3-2).

Maximum emergence of perennial seedlings, $63 \pm 5 - 77 \pm 9 \%$, occurred in the shallow mud treatment for all sources (Table 3-3). No perennial seedlings emerged in the deep sand treatment. Across sediment types, perennial seedlings were 4.7 times more likely to emerge at shallow (1 cm) compared to deep (5 cm) depths ($p < 0.001$; Tables 3-2

and 3-3). Across depths, perennial seedlings were 6.7 times more likely to emerge in mud compared to sandy sediments ($p < 0.001$; Tables 3-2 and 3-3).

Semi-annual seedling emergence was greatest, $19 \pm 7 \%$, in the shallow mud treatment (Table 3-3). Overall, increased depth significantly decreased emergence of semi-annual seedlings ($p < 0.001$; Tables 3-2 and 3-3). Semi-annual seedlings in shallower depth were 7.4 times more likely to emerge compared to seeds at deep depths (Table 3-2). Semi-annual seedling emergence was not significantly affected by sediment type ($p = 0.163$), however seedlings were 1.8 times more likely to emerge in mud compared to sandy sediments (Table 3-2).

Time to Seedling Emergence

The rate of seedling emergence for both perennial and semi-annual seeds increased over time (Figure 3-2). Mean time to seedling emergence was shorter for perennial (41 ± 0.9 to 50 ± 0.7 days) compared to semi-annual (48 ± 0.9 to 54 ± 0.3 days) seeds (Figure 3-2). For both semi-annual and perennial seed sources mean time to seedling emergence could not be calculated for the deep sand treatment due to a lack of emergence. For perennial seeds time to seedling emergence was affected by both sediment type ($p < 0.001$) and burial depth ($p < 0.001$; Table 3-4). Time to seedling emergence was 33 % longer for seeds in sandy sediment compared to muddy sediment and was 10 % longer for seeds in deep compared to shallow depths. Seed source also significantly affected time to seedling emergence of Chesapeake Bay seeds collected from the high organic sediment site (CB1) compared to North Carolina seeds collected from the low organic sediment site (NC2) seeds ($p < 0.001$). Time to seedling emergence

was 16 % longer for CB2 seeds. There was no significant difference in time to seedling emergence between Chesapeake Bay seeds collected from the low organic site (CB1) and NC2 seeds ($p = 0.261$). Time to emergence of semi-annual seedlings was significantly affected by depth ($p = 0.002$) with seeds in the deep treatments taking 26 % longer to emerge (Table 3-4). Sediment type had no significant effect on time to emergence for semi-annual seedlings ($p = 0.134$; Table 3-4).

Remaining Seed viability

Viability of non-emerged seeds at the conclusion of the 61 day experiment was low for both perennial and semi-annual seed sources (Table 3-3). Seed viability ranged from 0 ± 0 % to 9 ± 3 % in perennial treatments and from 0 ± 0 % to 5 ± 1 % in semi-annual treatments (Table 3-3). No treatment effects or interactions between treatments had a significant effect on either semi-annual or perennial seed viability (Table 3-5).

There was a large range of pre-emergent perennial (5 ± 1 % to 56 ± 13 %) and semi-annual seeds (5 ± 4 % to 17 ± 1 %; Table 3-3). For perennial seeds burial depth significantly effected the number of pre-emergent seeds with seeds at deeper depths 29 % more likely to split the seed coat but not emerge from the sediment surface than seeds at shallow depths ($p = 0.001$, Table 3-5). Chesapeake Bay seeds collected from the low organic sediment site (CB2) had significantly greater numbers of pre-emergent seeds compared to seeds collected from the low organic site in North Carolina (NC2; $p = 0.025$; Table 3-5). Seeds collected from the high organic sediment sites in the Chesapeake Bay (CB1) and North Carolina (NC2) were not significantly different ($p = 0.195$; Table 3-5). Sediment type did not have a significant effect on pre-emergence of perennial seeds

($p = 0.343$; Table 3-5). Semi-annual seed pre-emergence was not significantly affected by sediment type ($p = 0.149$) or burial depth ($p = 0.298$; Table 3-5). No interactions between treatments were significant for either semi-annual or perennial seeds (Table 3-5).

Chesapeake Bay seeds collected from the high organic sediment site (CB1) had the smallest proportion of non-viable seeds in the deep mud treatment ($3 \pm 1\%$) and Chesapeake Bay seeds collected from the low organic site (CB2) had the greatest proportion of non-viable seeds in the deep sand treatment ($48 \pm 5\%$; Table 3-3). Despite the large range of non-viable seeds between seed sources, seed source did not have a significant effect on the number of non-viable seeds ($p = 0.447$ CB1; $p = 0.238$ CB2; Table 3-5). Perennial seeds in sandy sediments ($p = 0.005$) and seeds in the deep burial depth treatments ($p = 0.001$) had significantly greater numbers of non-viable seeds compared to muddy shallow treatments (Table 3-5). Between $61 \pm 8\%$ and $83 \pm 6\%$ of all non-emergent semi-annual seeds were non-viable at the end of the experiment compared to $3 \pm 1\%$ and $48 \pm 5\%$ of perennial seeds (Table 3-3). The proportion of non-viable semi-annual seeds was similar across all treatments and neither sediment type ($p = 0.117$) nor burial depth ($p = 0.760$) had a significant effect on the number of non-viable seeds remaining at the end of the experiment (Table 3-5).

Seedling Biomass

Seed source had a significant effect on mean seedling biomass ($p < 0.001$). Post hoc analysis with Tukey's test indicated that mean initial biomass of perennial seedlings was greater than semi-annual seedlings ($p < 0.001$) and that there was no significant difference in biomass between perennial sources (Figure 3-3). Seedlings from muddy

treatments had significantly greater biomass compared to sandy treatments ($p < 0.001$). Burial depth also significantly affected mean seedling biomass as seedlings from seeds planted in deeper depths in muddy sediments had greater biomass than seedlings from seeds planted at the shallow depths ($p < 0.001$). There was also a significant interaction between sediment type and burial depth ($p < 0.001$) and among sediment type, burial depth, and seed source ($p = 0.035$).

Discussion

In this study perennial *Zostera marina* seeds had greater overall seedling emergence, shorter time to seedling emergence, and greater initial seedling biomass compared to semi-annual seeds. As both semi-annual and perennial seeds were exposed to the same environmental conditions, the lack of emergence in semi-annual seeds may be a reflection of the generally smaller seed size or in differing reproductive strategies between these semi-annual and perennial populations. In addition, similar responses between Chesapeake Bay and North Carolina perennial seed sources signify that regional differences between source beds was not the driving factor behind differing seedling emergence responses. Of the environmental factors investigated here depth was the only condition that affected both perennial and semi-annual seeds with maximum seedling emergence of both forms occurring at shallow (1 cm) depths. While maximum emergence and minimum time to emergence of perennial seedlings occurred in the muddy (3 % organic content) treatments, there was no significant difference in semi-annual seedling emergence between sediment types. Remaining seed viability was low

for all treatments indicating that non-germinated seeds may not contribute to a long-term sediment seed-bank.

Maximum seedling emergence of perennial seed sources ($77 \pm 9 \%$) was significantly greater compared to semi-annual seeds ($19 \pm 7 \%$). This result was unexpected as the semi-annual beds rely completely on seedling emergence for bed re-establishment. In general, perennial *Z. marina* beds allocate less energy to sexual reproduction compared to annual *Z. marina* by producing fewer flowering shoots (Phillips et al., 1983a; Keddy, 1987; Olsen, 1999). To compensate for the greater production of reproductive material, annual *Z. marina* shoots significantly reduce below ground production (Keddy, 1987). As a result, annual plants do not have the same nutritive reserves provided by rhizomes to perennial plants during the period of sexual reproduction (Harrison, 1979). Conditions of the parent plant during seed production have significant effects on seed size, seed viability, seed germination, and seedling emergence (Baskin and Baskin, 1998). Variation in *Z. marina* seed size has been documented between populations (Wyllie-Echeverria et al., 2003) with larger more robust seeds produced under environmental conditions that are more favorable for adult plant growth and survival (Baskin and Baskin, 1998). For this study *Z. marina* seeds from the semi-annual bed were notably smaller in width and length compared to those from perennial beds. Larger heavier *Z. marina* seeds may have a greater chance at germination (Luckenbach and Orth, 1999) and seedling establishment (Churchill, 1992) compared to smaller seeds. Therefore, the lower seedling emergence rates of the semi-annual *Z. marina* bed seeds presented in this study may be the result of a smaller seed size rather than environmental conditions.

Low emergence rates may also be compensated for by producing greater numbers of smaller seeds. Increased production of flowering shoots by annual populations results in seed production up to seven times greater than perennial populations (Keddy, 1987). As a spatial “bet-hedging” strategy smaller more easily dispersed seeds are capable of escaping stressful location conditions associated with annual autotrophic populations (Symonides, 1988; Shipley et al., 1989; Rees, 1996). By producing greater densities of seeds, annual populations increase the chances of more seeds finding suitable germination sites which also increase the odds of successful seedling establishment (Keddy, 1987; Harrison, 1993). For example, Santamaría-Gallegos et al. (2000) reported annual *Z. marina* populations in the Gulf of California produce between 2,334 shoots m⁻² and 30,000 seeds m⁻². Assuming initial seed viabilities similar to those found in this study (60 – 80 %) the number of viable seeds would be between 18,000 and 24,000. To reach reported shoot densities the maximum emergence rate would range between 9.7 and 12.9 % which is well within the emergence rates observed in this study of 5 – 19 %. Therefore, the overall low emergence rates from semi-annual bed *Z. marina* seeds may result in the sufficient number of seedlings required to re-establish annual or semi-annual beds yearly.

In this study perennial *Z. marina* seeds had significantly greater seedling emergence in sediments with 3 % organic content compared to sediments with < 1% organic content. Similar seedling emergence responses were reported in the Dutch Wadden Sea where perennial seed germination was greater in muddy sediments (2.2 ± 0.4 % organic content) compared to sandy sediments (1.0 ± 0.0 %; van Katwijk and Wijgertgangs, 2004). Due to the overall lack of emergence in either sediment type,

sediment organic content did not significantly affect emergence of semi-annual seedlings in the results presented here. Increases in sediment organic content reflect shifts in grain size (Koch, 2001), nutrient availability (Touchette and Burkholder, 2000; Tamaki et al., 2002), and oxygen concentration (Moore et al., 1993) signifying large changes in the sediment microenvironment. Small scale (order of mm to cm) changes in the surrounding sediment microenvironment have significant effects on seed germination responses (Harper, 1977). Diverging seedling emergence responses by semi-annual and perennial seeds to the sediment environment highlights the need for further analysis on the effects of edaphic factors on germination of semi-annual *Z. marina* seeds.

Greater overall perennial *Z. marina* seedling emergence in muddy compared to sandy sediments has been attributed to anoxic conditions resulting from a reduced environment (Moore et al., 1993; Probert and Brenchly, 1999; Terrados et al., 1999). However, in this study redox values were not significantly different between sediment sources and did not reach anoxic values at depths at which the seeds were planted. In addition to more reduced conditions muddy sediments are characterized by greater concentrations of inorganic phosphate and ammonia than sandy sediments (Thayer et al., 1984; Touchette and Burkholder, 2000; Koch, 2001; Tamaki et al., 2002). In this study the NH_4^+ concentrations were significantly greater in the muddy compared to sandy treatments. While the effects of nutrients on *Z. marina* seed germination is unknown, seeds of terrestrial plants are capable of N uptake prior to germination and the absence of or supersaturated concentrations of DIN in the sediment inhibit germination for many terrestrial species (Hilhorst and Karssen, 2000). Quantifying the effects of sediment NH_4^+ concentrations on perennial and semi-annual *Z. marina* bed seed germination and

seedling emergence was beyond the scope of this research; however, these results indicate that nutrient seed germination/emergence interactions require further research.

Aerobic sediment conditions may partially explain the delay in seedling emergence for both perennial and semi-annual bed seed sources. In previous laboratory studies perennial *Z. marina* seedling emergence was initiated within days of temperatures reaching 15 °C under anoxic conditions and was delayed for a period up to three months at the same temperatures under aerobic conditions (Moore et al., 1993). For this study water column temperatures were maintained at 14.2 ± 0.8 °C yet mean time to emergence ranged between 37 ± 1 days and 46 ± 2 days for perennial seeds and 48 ± 1 days and 54 ± 0 days for semi-annual seeds. The lack of an anoxic cue did not inhibit emergence completely, rather it was delayed increasing the time the embryo is completely reliant on the hypocotyl energy reserves (Taylor, 1957). The delay in seedling emergence for both seed types may have favored perennial bed seeds which, in general, were larger and may contain larger starch reserves (Harper, 1977; Wyllie-Echeverria et al., 2003).

Both perennial and semi-annual *Z. marina* seeds buried at 1 cm showed significantly greater emergence compared to seeds buried at 5 cm depths. These results are similar to Granger et al. (2000) who concluded that perennial *Z. marina* seeds buried deeper than 2 cm resulted in significantly lower seedling emergence. The lack of emergence of seeds buried at deeper depths may be related to hypocotyl elongation. The length of hypocotyl elongation is directly related to burial depth and the deeper burial depths may have delayed the emergence of the seedling cotyledon from the sediment surface, therefore cutting off the necessary oxygen required for survival (Churchill, 1992). This trend is reflected in the significantly greater number of pre-emergent

perennial seeds found in the 5 cm, burial treatments in both sediment types. Although the cues were present for the embryo to split the seed coat, *Z. marina* seeds may not have the required reserves necessary to produce seedlings at depths of 5 cm therefore they did not develop into seedlings. There was no significant difference in the number of pre-emergent semi-annual *Z. marina* seeds with depth. However, this may be reflective of the overall low germination characteristic of semi-annual seeds in this study.

The low viability of the remaining semi-annual (0 – 9 %) and perennial (0 – 5 %) seeds support the observations that both perennial and semi-annual bed *Z. marina* seeds do not form long-term seed-banks (Orth et al., 2000; Jarvis Chapter 2). Laboratory results show that *Z. marina* seeds can remain viable for 8 to 12 months after release from flowering shoots when kept in water under aerated conditions (Lamounette, 1977; Orth et al., 2007). Field experiments with perennial and semi-annual *Z. marina* seeds placed in the sediment reported similar results with a time period for maximum *Z. marina* seed viability of < 12 months (Jarvis, Chapter 2). Seeds play a large role in the recovery of perennial beds from large scale declines (Plus et al., 2003; Greve et al., 2005) and in the maintenance and re-establishment of annual beds (Phillips et al., 1983a; Santamaría-Gallegos et al., 2000). Low viability of seeds remaining in the sediment may have long-term implications for the maintenance and survival of both perennial and semi-annual *Z. marina* populations.

Initial seedling biomass was significantly affected by both sediment type and burial depth for semi-annual and perennial *Z. marina* sources. Seedling biomass was greater in the muddy compared to sandy treatments suggesting that sediment conditions that were most conducive to *Z. marina* seed germination also enhanced initial seedling

establishment. Muddy sediments with fine textures are generally more nutrient enriched compared to sandy sediments (Thayer, 1984; Koch, 2001) and as *Z. marina* absorbs the majority of NH_4^+ through its roots and rhizomes (Touchette and Burkholder, 2000) the muddy sediments may have provided greater NH_4^+ to fuel seedling growth.

Experimental additions of N based fertilizers to *Z. marina* seedlings under laboratory conditions resulted in greater growth (recorded as leaf length) in those treatments exposed to N fertilization compared to the control plots (Roberts et al., 1984). In addition, differences between perennial and semi-annual seedlings may have been a factor of time as perennial seeds germinated on average 17 days earlier than semi-annual seeds. This may have provided a substantial time advantage for initial seedling growth resulting in greater biomass in perennial compared to semi-annual seedling biomass.

In this study perennial bed *Z. marina* seeds, regardless of source, had greater overall emergence, shorter time to emergence, and greater initial biomass compared to semi-annual seeds. The results reported here suggest differing strategies between western mid-Atlantic semi-annual and perennial *Z. marina* populations for successful seedling establishment. Semi-annual populations use a “bet hedging” strategy producing smaller, less fit seeds that are able to disperse to more “safe sites” and escape local stressful conditions while perennial populations produce fewer, larger seeds that have higher emergence rates increasing their competitive fitness within the more stable perennial bed. While there was no significant regional difference in seedling emergence between perennial *Z. marina* sources sediment conditions did significantly affect seedling emergence, highlighting the role of the surrounding sediment rather than the location of the source population on successful sexual reproduction within mid-Atlantic perennial *Z.*

marina beds. Reduced seedling emergence of both semi-annual and perennial *Z. marina* seeds at burial depths > 1 cm may represent a possible bottleneck in successful *Z. marina* sexual reproduction. Implications of reduced *Z. marina* seed viability due to burial depth or sediment conditions may affect the resiliency to and recovery from disturbance for both perennial and semi-annual *Z. marina* beds.

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Table 3-1. Sediment characteristics for the sand and mud treatments. All values are mean \pm S.E.

	Sand		Mud	
	1 cm	5 cm	1 cm	5 cm
% Organic	0.5 \pm 0.0	0.4 \pm 0.0	3.1 \pm 0.1	3.5 \pm 0.3
Sand:Silt:Clay				
% Sand	96.5 \pm 0.1	96.7 \pm 0.4	42.0 \pm 0.2	41.3 \pm 0.9
% Silt	1.6 \pm 0.1	0.7 \pm 0.2	42.8 \pm 0.2	44.2 \pm 0.3
% Clay	1.9 \pm 0.2	2.6 \pm 0.1	15.2 \pm 0.1	14.5 \pm 0.8
Shepard's Index	sand	sand	sandy silt	sandy silt
Nutrients				
NH ₄ ⁺ μ M	18.2 \pm 4.1	33.1 \pm 3.8	32.5 \pm 2.3	102.6 \pm 30.7
PO ₄ ⁻³ μ M	0.3 \pm 0.3	0.2 \pm 0.1	0.0 \pm 0.0	0.0 \pm 0.0

Table 3-2. Logistic regression results and odds ratios for the effects of seed source, sediment type, and burial depth on maximum semi-annual and perennial seedling emergence. All significant results are denoted with an (*).

Parameter	DF	Estimate	SE	Wald 95 % CL		Chi Sq	<i>p</i>	odds ratio	Wald 95 % CL	
				Low	High				Low	High
Perennial										
Intercept	1	-1.79	0.19	-2.16	-1.42	89.78	<0.001*	--	--	--
Source – CB1	1	0.41	0.19	0.04	0.78	4.66	0.031*	1.50	1.04	2.18
Source – CB2	1	-0.60	0.19	-0.97	-0.22	9.66	0.002*	0.55	0.38	0.80
Source – NC2	0	--	--	--	--	--	--	--	--	--
Sediment	1	1.90	0.16	1.58	2.22	133.62	<0.001*	6.67	4.83	9.21
Depth	1	1.54	0.16	1.22	1.86	89.26	<0.001*	4.68	3.40	6.45
Semi-annual										
Intercept	1	-3.92	0.57	-5.04	-2.81	47.27	<0.001*	--	--	--
Sediment	1	0.57	0.41	-0.23	1.39	1.95	0.163	1.78	0.79	4.00
Depth	1	2.00	0.55	0.91	3.08	13.05	<0.001*	7.38	2.50	21.84

Table 3-3. A. Maximum emergence and mean time to emerge across all treatments. B. Viability results across all treatments. All values are mean \pm S.E.

Source	Sediment	Depth (cm)	A. Germinated Seeds		B. Non-Germinated Seeds		
			MTG (days)	Max % Germ	% Viable	% Non-Viable	% Pre-Emerge
Perennial							
CB1	Mud	1	37 ± 1	77 ± 9	3 ± 1	15 ± 8	5 ± 1
		5	46 ± 1	75 ± 5	1 ± 1	3 ± 1	21 ± 5
	Sand	1	49 ± 1	73 ± 6	1 ± 1	17 ± 4	9 ± 5
		5	--	0 ± 0	9 ± 3	36 ± 10	56 ± 13
CB2	Mud	1	44 ± 2	64 ± 8	1 ± 1	21 ± 7	13 ± 5
		5	46 ± 1	57 ± 7	1 ± 1	8 ± 0	33 ± 7
	Sand	1	54 ± 1	29 ± 5	5 ± 4	24 ± 0	41 ± 7
		5	---	0 ± 0	5 ± 3	48 ± 5	45 ± 6
NC2	Mud	1	41 ± 1	63 ± 5	0 ± 0	21 ± 6	16 ± 2
		5	46 ± 2	64 ± 5	3 ± 3	9 ± 1	24 ± 2
	Sand	1	47 ± 1	68 ± 2	7 ± 7	15 ± 7	12 ± 4
		5	---	0 ± 0	5 ± 4	43 ± 4	52 ± 7
Semi-Annual							
NC1	Mud	1	48 ± 0.9	19 ± 7	3 ± 3	61 ± 8	17 ± 1
		5	54 ± 0.3	5 ± 1	4 ± 0	75 ± 9	15 ± 7
	Sand	1	51 ± 0.6	15 ± 4	0 ± 0	80 ± 2	5 ± 4
		5	---	0 ± 0	5 ± 1	83 ± 6	12 ± 6

Table 3-4. Survival analysis results of the effects of seed source, sediment type, and burial depth on time to emergence of perennial and semi-annual seeds. All significant results are denoted with an (*).

Parameter	DF	Estimate	SE	Wald 95 % CL		Chi Sq	P
				Low	High		
Perennial							
Intercept	1	3.97	0.05	3.88	4.06	7384.59	<0.001*
Source - CB1	1	-0.05	0.04	-0.13	0.03	1.27	0.261
Source - CB2	1	0.15	0.04	0.07	0.24	12.46	<0.001*
Source - NC2	0	--	--	--	--	--	--
Sediment	1	-0.55	0.04	-0.62	-0.48	216.03	<0.001*
Depth	1	0.10	0.01	0.08	0.12	118.90	<0.001*
Scale	1	0.44	0.02	0.41	0.48		
Semi-Annual							
Intercept	1	4.53	0.24	4.07	5.00	365.89	<0.001*
Depth	1	0.24	0.08	0.09	0.39	10.11	0.002*
Sediment	1	-0.29	0.20	-0.68	0.09	2.24	0.134
Scale	1	0.46	0.08	0.33	0.65		

Table 3-5. Poisson regression results for remaining (A) viable, (B) non-viable, and (C) pre-emergent seeds. All significant results are denoted with an (*).

Parameter	A. Non-Germinated Viable					B. Non-Germinated Non-Viable					C. Pre-Emergent Non-Viable				
	DF	Est	SE	X ²	p	DF	Est	SE	X ²	p	DF	Est	SE	X ²	p
Viable															
Perennial															
Intercept	1	0.08	0.99	0.01	0.934	1	0.76	0.44	2.96	0.085	1	0.95	0.41	5.37	0.021*
Source - CB1	1	-0.92	1.53	0.36	0.549	1	0.44	0.57	0.58	0.447	1	-0.83	0.64	1.68	0.195
Source - CB2	1	0.16	1.36	0.01	0.908	1	0.63	0.53	1.39	0.238	1	1.06	0.47	5.02	0.025*
Source - NC2	0	--	--	--	--	0	--	--	--	--	0	--	--	--	--
Sediment	1	-1.31	1.52	0.74	0.389	1	1.34	0.48	7.91	0.005*	1	-0.46	0.49	0.90	0.343
Depth	1	0.08	0.22	0.14	0.707	1	0.27	0.08	11.54	0.001*	1	0.25	0.08	11.06	0.001*
Sed.*CB1	1	0.64	1.31	0.24	0.624	1	-0.68	-0.48	1.99	0.159	1	-0.41	0.38	1.16	0.282
Sed.*CB2	1	0.12	1.38	0.01	0.929	1	-0.42	0.42	1.01	0.315	1	-0.13	0.35	0.14	0.713
Sed.*NC2	0	--	--	--	--	0	--	--	--	--	0	--	--	--	--
Depth*CB1	1	0.16	0.31	0.28	0.597	1	-0.11	0.11	0.98	0.321	1	0.16	0.12	2.04	0.153
Depth*CB2	1	-0.07	0.29	0.05	0.815	1	-0.08	0.96	0.76	0.384	1	-0.16	0.09	3.37	0.067
Depth*NC2	0	--	--	--	--	0	--	--	--	--	0	--	--	--	--
Sed.*Depth	1	-0.05	0.28	0.03	0.854	1	-0.48	0.10	24.92	<0.001*	1	-0.01	0.08	0.00	0.956
Dispersion	1	0.29	0.44	--	--	1	-0.05	0.03	--	--	1	-0.01	0.03	--	--
Semi-Annual															
Intercept	1	-1.94	1.42	1.86	0.173	1	2.98	0.12	581.88	<0.001*	1	-0.12	0.99	0.01	0.905
Sediment	1	1.24	1.71	0.53	0.468	1	-0.35	0.22	2.46	0.117	1	1.67	1.16	2.09	0.149
Depth	1	0.36	0.25	2.08	0.150	1	0.01	0.03	0.09	0.760	1	0.20	0.20	1.09	0.298
Sed.*Depth	1	-0.23	0.31	0.56	0.456	1	0.04	0.05	0.80	0.372	1	-0.24	0.24	1.05	0.306
Dispersion	1	-0.50	0.00	--	--	1	-0.04	0.01	--	--	1	0.04	0.24	--	--

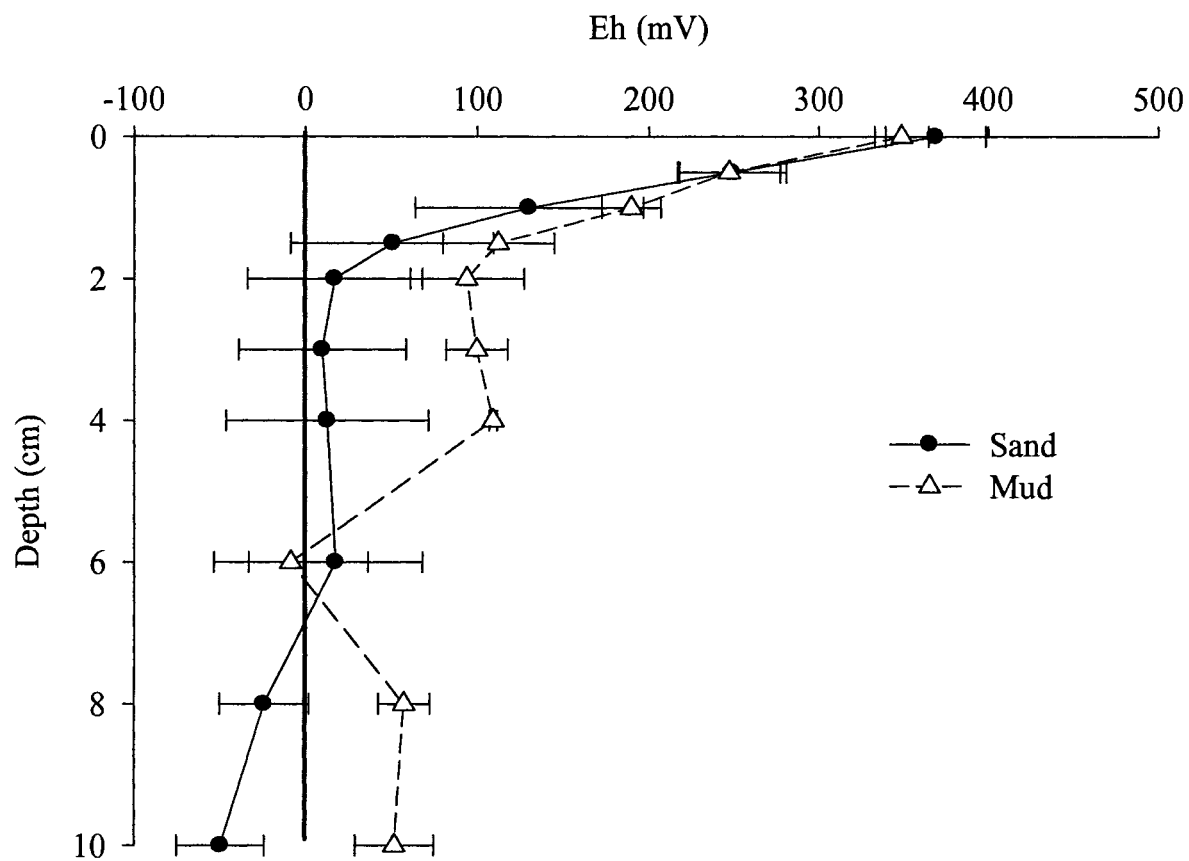


Figure 3-1. Mean vertical redox (Eh) measurements from cores collected from all treatments at the end of the experiment. Values are mean \pm SE.

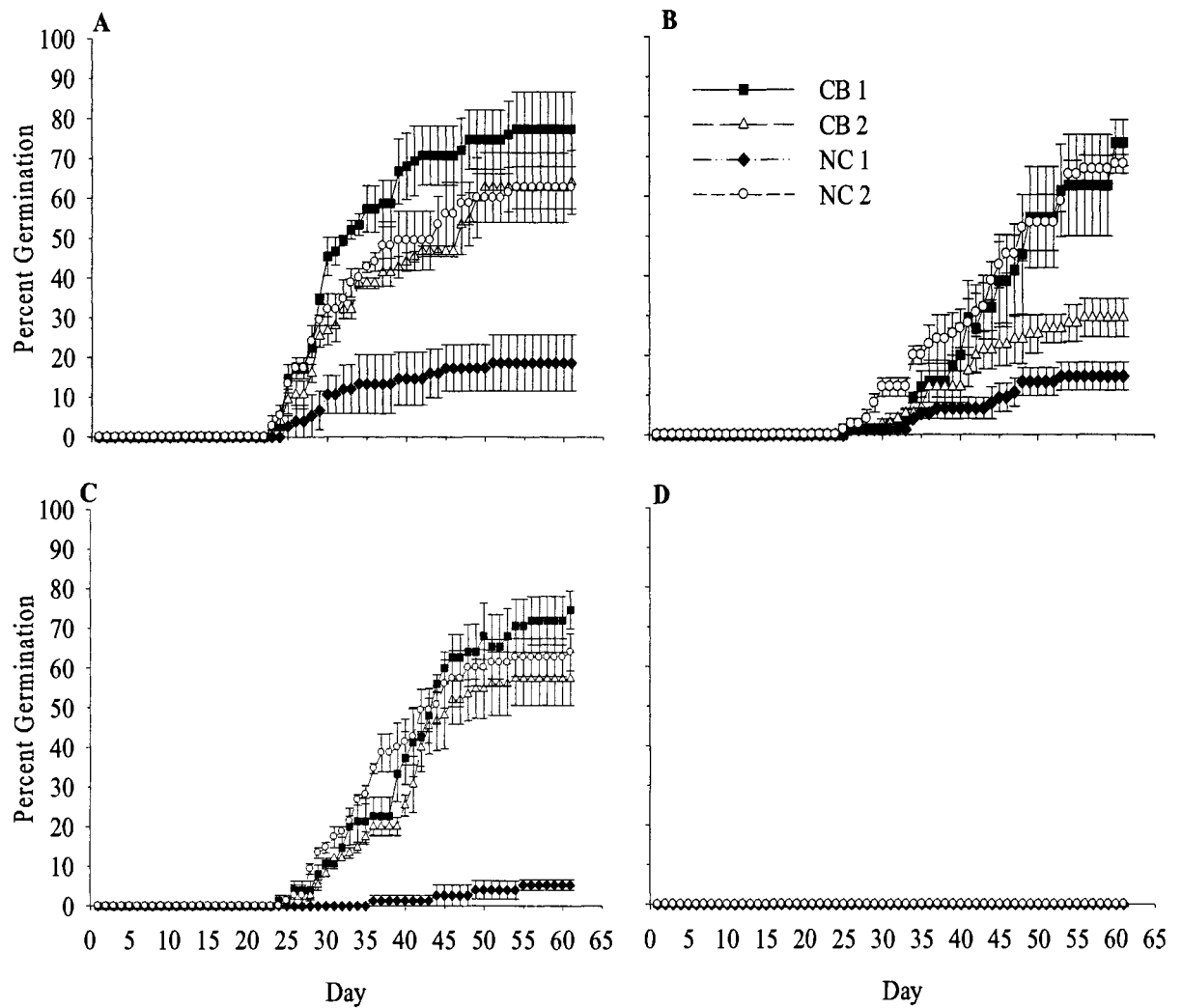


Figure 3-2. Cumulative mean percent germination curves for (A) mud 1 cm; (B) sand 1 cm; (C) mud 5 cm; (D) sand 5 cm treatments. Values are mean \pm S.E.

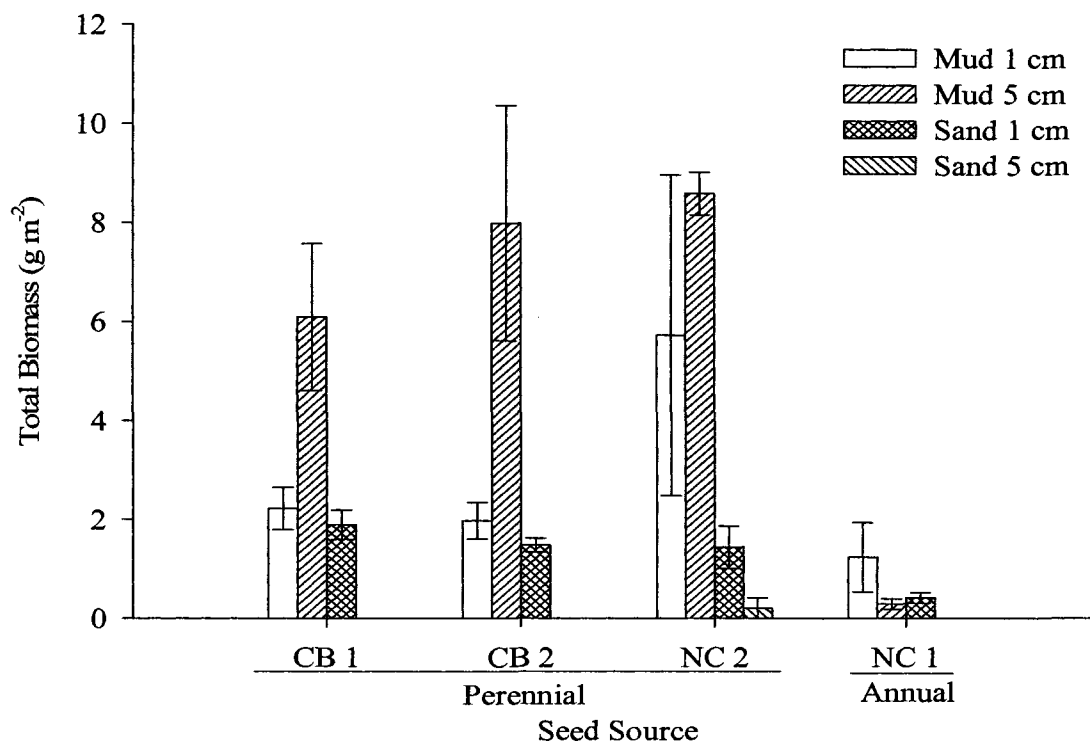


Figure 3-3. Mean total *Z. marina* seedling biomass. Bars are mean \pm standard error.

CHAPTER 4 : POTENTIAL LONG-TERM LOSS OF ZOSTERA MARINA BEDS IN
THE CHESAPEAKE BAY FOLLOWING REPEATED DISTURBANCE EVENTS:
THE ROLE OF SEEDLINGS AND SEED-BANK VIABILITY.

Abstract

Perennial *Zostera marina* beds in two spatially separated regions of the York River were sampled for changes in *Z. marina* abundance from 2004 to 2008. In the summer of 2005 large declines in perennial *Z. marina* populations occurred throughout the lower Chesapeake Bay, including these areas. From April – October 2006 and 2007 monthly sampling of shoot origin (seedling or vegetative) and the density and viability of seeds within the seed-bank was conducted to characterize bed re-development. In the spring of 2006 *Z. marina* beds in both regions re-established with seedlings providing 48 – 54 % of the total shoot density. Few flowering shoots and no viable seeds in the sediment were recorded. In 2007 vegetative shoots were dominant with new seedlings comprising only 0 – 6 % of total shoot density. A second consecutive decline occurred during the summer of 2006 in the upriver region. Recovery in this region was minimal in 2007 with maximum shoot density of only 6 ± 6 shoots m^{-2} compared to 526 ± 59 shoots m^{-2} in the downriver region. These results demonstrate the importance of seedlings in initial bed recovery following a single disturbance, but highlight the sensitivity of *Z. marina* beds in this region to repeated stresses. After a single disturbance event few viable seeds remain in the sediment following seedling germination. Since seedlings in this region during their first year of growth are not observed to flower and produce seeds, the seed-bank is not immediately replenished and there is limited capacity for bed re-growth. With this loss of resiliency a repeated disturbance can result in nearly complete bed loss.

Key Words: *Zostera marina*, decline, seedling, seed-bank

Introduction

In 2005 *Zostera marina* populations suffered a sudden and near complete die-back across the lower Chesapeake Bay (Moore and Jarvis, 2008). By comparing water quality and *Z. marina* monitoring data from the York River, a tributary to the Chesapeake Bay, the decline was related to a period of unusually high water temperatures in July and August 2005. These stressful water temperatures combined with low light conditions resulted in a large scale decline over a short period of only two months. While *Z. marina* populations have been declining gradually throughout the Chesapeake Bay region over the last 80 years (Orth and Moore, 1983; Moore et al., 2000) sudden declines in response to episodic stress events have not been reported since Tropical Storm Agnes in 1972 (Orth, 1976; Orth and Moore, 1983, 1984; Moore et al., 2000). Therefore patterns or mechanisms of recovery in these beds are uncertain.

Growth and survival of adult *Z. marina* plants are significantly affected by the surrounding environment both in the water column and in the sediment (Dennison et al., 1993). *Z. marina* mortality can increase 12 fold when water temperatures are between 25 and 30 °C compared to mortality rates at temperatures ranging from 10 to 20 °C (Nejrup and Pederson, 2008). During the summer of 2005 York River water temperatures reached 33 °C over short time periods resulting in the removal of the majority of above ground biomass (Moore and Jarvis, 2008). In *Z. marina* beds where damage to the bed by fishing gear (Neckles et al., 2005) or disease (Frederiksen et al., 2004) does not result in the complete removal of above ground biomass, beds are estimated to recover over a time period of years to decades through rhizome elongation (Neckles et al., 2005). In cases, such the one documented in the Chesapeake Bay in 2005, where nearly all of the

above ground biomass is lost the re-establishment mechanism may not be dependent upon vegetative growth but rather on seed germination and seedling establishment (Plus et al., 2003; Greve et al., 2005).

Sudden die-offs of *Z. marina* populations in response to episodic stress have been documented in both France and Denmark (Plus et al., 2003, Greve et al., 2005). In the Thau lagoon in the French Mediterranean Sea and the Odense Fjord in Denmark, large scale declines in *Z. marina* populations occurred after prolonged periods of complete anoxic conditions (Plus et al., 2003; Greve et al., 2005). Following the defoliation event at both sites the initial recruitment into the degraded area was accomplished by seed germination and seedling survival where between 80 % and 96 % of total shoots were surviving seedlings (Plus et al., 2003; Greve et al., 2005). Only after this initial re-establishment did asexual reproduction, the dominant mode of reproduction for perennial *Z. marina* (den Hartog, 1970; Tomlinson, 1974), play a role in the recovery process as the expansion of the beds and increases in biomass were due to vegetative growth. Recovery periods were recorded on time scales of months to years (Plus et al., 2003).

Unlike perennial *Z. marina* beds which rely on asexual reproduction for growth and persistence (Setchell, 1929; den Hartog, 1970), annual beds rely on seed germination and seedling establishment for bed re-establishment on an annual basis (Keddy and Patriquin, 1978; Phillips et al., 1983). By surviving as a seed in the sediment seed-bank during potentially stressful times of the year, annual *Z. marina* populations are able to grow and reproduce in areas where annual loss of above ground biomass to extreme conditions such as water temperatures > 30 °C (Meling-Lopez and Ibarra-Obando, 1999; Santamaría-Gallegos et al., 2000) and physical removal by ice (Keddy and Patriquin,

1978; Robertson and Mann, 1984) or grazers (den Hartog, 1970) inhibits the persistence of perennial beds (Keddy and Patriquin, 1978). For many aquatic plant species the role of the seed-bank within the population increases in importance in direct relation to the type of disturbance (Combroux et al., 2001). Although perennial beds are found in less disturbed areas than annual beds they still produce seed-banks; however, the production of seeds is extremely variable and not well understood (Orth et al., 2000).

Seeds need to remain viable in the sediment for the seed-bank to serve any function (Leck et al., 1989). In the Chesapeake Bay, *Z. marina* reproductive shoots and subsequent seed production are extremely patchy with flowering shoots making up between 1 % and 88 % of total shoots in a 0.018 m⁻² core (Harwell and Orth, 2002), resulting in the production of 50 – 200 seeds per m⁻² (Harwell and Rhode, 2007). As a result of variable production, seed-banks are also extremely patchy with extrapolated seed densities ranging from 55 to 6,160 seeds m⁻² and viability ranging from 40 to 58 % during an average year (Harwell and Orth, 2002). In addition, Chesapeake Bay *Z. marina* plants do not produce a long term viable seed-bank as viability decreases significantly after only 6 months in the sediment and is < 1 % after one year (Orth et al., 2000; Jarvis Chapter 2).

The objective of this study was to quantify the spatial and temporal recolonization characteristics of *Z. marina* beds in the York River, a tributary to the Chesapeake Bay, for two years immediately following the 2005 decline. Specifically, percent cover, density, and biomass of both vegetative and reproductive shoots for both seedlings and surviving vegetative shoots were quantified. In addition, I estimated sediment seed-bank reserves to determine how seeds could contribute to the resiliency of established *Z.*

marina beds in the York River subjected to multiple disturbances. Finally, in order to gain a better understanding of the interactions between environmental conditions and natural *Z. marina* re-establishment both water column and sediment characteristics were quantified while monitoring recolonization patterns.

Methods

Site Selection

Three beds were chosen in the lower York River based on the documented decline of *Z. marina* shoots in the fall of 2005 (Jarvis and Moore, 2008). Two downriver beds were located at the mouth of the York River at the Goodwin Island National Estuarine Research Reserve (GI1, GI2; 37° 13' N; 76° 23' W; Figure 4-1). The third bed was located 10 km upriver adjacent to the Virginia Institute of Marine Science, Gloucester Point campus (GP; 37° 14.8' N, 76° 30.3' W; Figure 4-1). All three beds have been monitored for interannual variation in cover and density since 2004 (www.nerrs.noaa.gov/monitoring/biological.html).

Three transects running parallel to shore were established at each bed. Transects were placed in the inner, middle, and outer edges of the bed and varied in placement and length depending on bed size. On average, the GP and GI2 beds were 100 m and 130 m wide, respectively. At these beds the inner transect was placed 20 m from the shore, the mid-bed was placed at 50 m, and the outer edge was placed at 70 m. The GI1 bed was 700 m wide and transects were placed at 100 m, 300m, and 500 m from the shore, respectively. Transects at the GP and GI2 beds were 30 m long while all transects at GI1 were 50 m long. Within each transect 6 or 12 (depending on transect length) randomly

selected 1 m⁻² permanent quadrats were established to monitor changes in *Z. marina* density and cover within the bed.

Sediment Characterization

Prior to sampling and during each sampling period, three sediment cores (11.4 cm diameter by 10 cm depth) were collected from all transects, sectioned into 0-5 cm vertical sections, and quartered. The first quarter was sieved (63 µm sieve), washing slit and clay fractions into a graduated cylinder. After 24 hours, pipette analysis was performed to determine the clay (8 phi) and silt (4 phi) fractions of the sample. Dry weights of the aliquots were compared and percent sand silt and clay fractions were determined (modification of Plumb, 1981). All sediments were classified based on sand/silt/clay ratios (Shepard, 1954).

Percent organic matter in the sediment was determined by drying two quarters of the sediment sub-sample at 60 °C for five days or until a constant dry weight was reached. Ten grams of the dried sediment sub-sample were weighed and combusted at 500 °C for five hours. The sample was then weighed again and percent organic matter was calculated (Erftemeijer and Koch, 2001). The fourth quarter of the sub-sample was analyzed to determine sediment exchangeable nutrients. Samples were extracted in 2 M KCl, shaken for 1 hour, centrifuged at 4000 rpm for 6 minutes, filtered (Gelman Supor, 0.45 µm), and frozen until analysis. Dissolved inorganic nitrogen (DIN – NH₄⁺) and dissolved inorganic phosphorous (DIP – PO₄⁻³) were determined in the samples using a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002). In addition, vertical redox (Eh) profiles of all

sediment types were measured with a 21 cm platinum electrode. The probe was inserted into the top of the core and redox was measured at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 cm. Final readings were corrected for temperature relative to the reference electrode (Hinchey and Schaffner, 2005).

Water Column Characterization

Water temperature ($^{\circ}\text{C}$), salinity (PSS), and dissolved oxygen (mg l^{-1}) were measured with a Yellow Spring Instruments, Inc. (YSI, Inc., Yellow Spring, Ohio) model 6600 EDS sonde at both the down river (Goodwin Island) and upriver sites (Gloucester Point). Water quality data were recorded from a depth of 25 cm above the bottom every 15 minutes for the duration of the study. In addition three water samples were collected monthly throughout the duration of the study from each site. Samples were filtered (Gelman Supor, $0.45\ \mu\text{m}$) and analyzed for DIN (NO_x , NO_2^- , NH_4^+) and DIP (PO_4^{3-}) with a Lachat auto analyzer (Liao 2001, revised 2002; Knepel and Bogren 2001, revised 2002; Smith and Bogren 2001, revised 2002). The remaining water samples were filtered and analyzed for chlorophyll *a* (Strickland and Parson, 1972) and total suspended solids (TSS). TSS was quantified from a well-mixed sample of known volume. The sample was filtered through a GF/F filter and the residue retained on the filter was dried to constant weight at $103 - 105\ ^{\circ}\text{C}$ and reported as $\text{mg total suspended solids l}^{-1}$. Ambient incident irradiance was measured with a LI-COR terrestrial sensor (LI-190SA) and light attenuation, or $K_d(\text{m}^{-1})$, to a depth of 1 m through the water column was quantified by measuring light levels beneath the water surface at each site with a LI-COR underwater light sensor (LI-192SA).

Recolonization Characteristics

To characterize vegetation at each site percent cover, vegetative shoot density, and reproductive shoot density were measured monthly from April to October in 2006 and 2007 within permanent one m⁻² quadrats randomly selected from each transect (6 quadrats for GI2 and GP; 12 quadrats for GI1). Percent cover was visually estimated and *Z. marina* vegetative shoot and reproductive shoot density were quantified within each quadrat.

Above and belowground biomass were also quantified at each site with a 22 cm diameter corer (n = 5). Samples were collected monthly at five random locations dispersed within five meters of each transect. All cores were sieved in the field (1 cm mesh) and plant and root/rhizome material were stored in York River water at 4-6 °C until analysis. All plants within each sample were identified as seedlings or vegetative shoots based on their rhizome structure and sectioned into above and below ground material with separation occurring at the basal meristem. In addition, all reproductive shoots, number of spathes per reproductive shoot, and the number of seeds per spathe were counted. All biomass samples were dried in pre-weighed aluminum envelopes for five days at 50 °C or until a constant dry weight was reached.

Sediment Seed-bank

Five additional cores (11.4 cm diameter by 10 cm depth) were collected monthly from all transects and divided into 2 cm sections in the field. The sediment was stored at 4 °C until analysis. Seeds were sieved from the sediment (0.5 mm mesh) and then tested for viability using the tetrazolium staining method (Lakon, 1949; AOSA 1981; Sawma

and Mohler, 2002). Seed embryos were removed from their seed coats and soaked in a 1 % tetrazolium chloride (tetrazolium) solution for 24 hours before examination on a dissecting scope at 10 x magnification.

Data Analysis

Differences in vegetative shoot density and biomass, reproductive shoot density and biomass, and sediment seed-bank density between transects and sites and over time were analyzed using negative binomial regression with time (year), transect, source (seedling or vegetative shoot), and site as factors (PROC GENMOD: SAS Institute Inc.; Allison, 1999). For all significant ($p < 0.05$) model terms odds ratios were calculated using Wald chi square statistics (SAS; SAS Institute Inc). Model selection for both semi-annual and perennial seed sources was based on log-likelihood estimates (Burnham and Anderson, 2002).

Temporal effects on percent cover were analyzed using repeated measures Analysis of Variance with time (year), transect, and site quantified as factors. (PROC GLM; SAS Institute Inc). The effect of source on cover was not quantified due to the non-destructive methods used to estimate percent cover. These methods did not allow for the rhizomes to be examined therefore source could not be quantified. Prior to analysis data were transformed with arcsine square root transformation, normality was confirmed, and homogeneity of variance was verified with Cochran's test (Zar, 1999).

The effect of bed and transect on the proportion of reproductive shoots produced were analyzed with non-parametric Kruskal-Wallis and Kolmogorov-Smirnov tests (The SAS System for Windows, SAS Institute Inc.) after the data were found to be non-normal

following transformation. Time (year) and source were not analyzed as reproductive shoots were not produced in 2006 and no seedlings produced reproductive shoots in 2007.

Differences in water column characteristics and sediment percent sand/silt/clay ratios between beds and over time were analyzed with nonparametric statistics as the data were non-normally distributed (Kruskal-Wallis and Kolmogorov-Smirnov Tests; The SAS System for Windows, SAS Institute Inc.). All remaining sediment data were transformed when necessary and analyzed with a repeated measure ANOVA to compare the effects of time, site and the interactions of these factors. Results are presented as non-transformed means \pm standard error of the mean.

Results

Environmental Characterization

All water column characteristics (temperature, salinity, chlorophyll *a*, TSS, K_d , DIN, DIP) followed seasonal trends and were significantly different over time ($p < 0.001$ for all). Water column temperature ($p = 0.291$) and salinity ($p = 0.848$) did not differ significantly between the upriver and down river sites in 2006 and 2007 (Figure 4-2A, B). Temperatures ranged from 13.6 ± 0.1 °C to 28.5 ± 0.1 °C and salinity ranged from 15.5 ± 0.1 to 23.2 ± 0.1 at both sites.

Water column DIN did not vary significantly with site (NO_x $p = 0.100$; NO_2^- $p = 0.105$; NH_4^+ $p = 0.117$) but did differ significantly over time ($p < 0.001$). All DIN concentrations averaged < 1 μM and did not reach concentrations above 8 μM during the 2006 and 2007 sampling season. Water column DIP was significantly different between

sites ($p < 0.001$) and over time ($p < 0.001$) although concentrations did not increase above $1 \mu\text{M}$ during the entire sampling period.

Water clarity (K_d to 1 m) was significantly greater at the downriver site compared to the upriver site ($p = 0.006$). At the upriver site chlorophyll *a* ranged from $8.13 \pm 0.39 \mu\text{g l}^{-1}$ to $26.80 \pm 5.89 \mu\text{g l}^{-1}$ which was significantly greater ($p = 0.002$) than the down river site where chlorophyll *a* ranged from $6.07 \pm 0.06 \mu\text{g l}^{-1}$ to $23.55 \pm 0.92 \mu\text{g l}^{-1}$. Total suspended solids did not differ significantly between sites ($p = 0.068$) and ranged from $6.27 \pm 1.05 \text{ mg l}^{-1}$ to $38.98 \pm 7.41 \text{ mg l}^{-1}$.

In the upriver site $< 22\%$ of light reached depths greater than 1 m in July and August 2006 and again from June to October 2007 (Figure 4-2 C). Light at the downriver site was reduced to below 22% of surface irradiance in August 2007 and the reduced light conditions persisted for < 1 month. The low light periods at the upriver site in both 2006 and again in 2007 coincided with seasonally high summer water temperatures (Figure 4-2).

All sediments were classified as sand ($> 75\%$ sand). Sediment percent organic content varied significantly between sites ($p < 0.001$) and transects ($p < 0.001$) but not over time ($p = 0.317$). *Post hoc* analysis with Tukey's test indicates that all sites were significantly different; however, only the shallow transects were significantly different than the mid-bed and deep transects. There was no significant difference in organic matter between the mid-bed and deep transects at all sites. The GI1 shallow transect contained the greatest organic content with an average value of $1.77 \pm 0.07\%$. Mean sediment organic content in both GI2 and GP shallow transects was $< 1\%$. The sediment organic content at the GP shallow transect averaged $0.85 \pm 0.13\%$ and GI2 averaged 0.53

± 0.03 %. Comparatively, sediment organic content in the mid-bed and deep transects averaged 0.57 ± 0.11 % for GI1, 0.42 ± 0.05 % for GP, and 0.43 ± 0.09 % for GI2.

Sediment NH_4^+ concentrations did not differ significantly between sites ($p = 0.257$) but were significantly different between transects ($p = 0.001$) and over time ($p < 0.001$). *Post hoc* analysis with Tukey's HSD test indicated that sediment NH_4^+ concentrations between the shallow and deep transects were significantly greater in the shallower compared to deep transects; however, there was no significant difference between the shallow and mid-bed or between mid-bed and deep transects. Tukey's HSD test also indicated significantly lower sediment NH_4^+ concentrations in April – July compared to August – September. There was a significant interaction between time and site ($p < 0.001$) and between time, site and transect ($p < 0.001$).

Sediment PO_4^{-3} concentrations varied significantly with site and time ($p < 0.001$ for both) but not across transects ($p = 0.142$). *Post hoc* analysis with Tukey's HSD test revealed that sediment PO_4^{-3} concentrations were significantly greater at GI2 than GI1 and GP but were not different between GI1 and GP. There was no clear trend with PO_4^{-3} concentrations over time as all months were significantly different from all other months. On average sediment PO_4^{-3} concentrations were < 1 μM for all sites and did not rise above 3.83 ± 0.84 μM throughout the monitoring period.

Eh was significantly different between sites ($p < 0.001$), across depths ($p < 0.001$) and over time ($p < 0.001$) but not between transects ($p = 0.065$). *Post hoc* analysis with Tukey's HSD test reveals that sediments were more reduced at the GI1 and GI2 sites compared to GP and at depths > 3 cm compared to shallower depths.

Pre-Divide vs. Post-Divide

During the 2004 – 2007 sampling period there was no significant difference in the mean *Z. marina* density between beds (Table 4-1). All sites declined suddenly in the fall of 2005 and across all sites in October density ranged from only 0 ± 0 to 29 ± 15 shoots m^{-2} (Figure 4-3). By July 2006 all sites were re-vegetated and shoot density ranged from 101 ± 23 to 228 ± 37 shoots m^{-2} . When compared to the 2004 sampling season, across site *Z. marina* density was significantly lower in all years (2005 $p = 0.004$; 2006 $p = 0.003$; 2007 $p < 0.001$) indicating that there was a long term negative effect of the 2005 decline on total shoot density (Figure 4-3). Following the 2005 decline shoot density was 1.8 times less in 2006 and 2.1 times less in 2007 compared to 2004 levels. Despite the lasting negative effect, *Z. marina* shoot densities in both the upriver and down river regions returned to pre-divide 2005 levels by 2006 ($p = 0.601$). Only the downriver sites remained at these levels during 2007 ($p = 0.811$) as a secondary decline occurred at the upriver GP site in the fall of 2006. This decline resulted in significantly lower shoot density at the upriver site compared to the downriver sites in 2007 ($p < 0.001$).

Total Cover

Overall, *Z. marina* cover was not significantly affected by position within the bed as there was no significant difference in cover between the shallow edge of the bed (closest to the shore), the mid-bed, and deep edge ($p = 0.083$). This trend was driven by the GI2 and GP sites where cover was similar across the shallow, mid-bed, and deep transects (Figure 4-4). Within the GI1 site cover was significantly greater at the shallow compared to mid-bed and deep sites ($p < 0.001$). Cover was also significantly affected by

site with cover at GI1 significantly greater than at GI2 and GP ($p < 0.001$). Time significantly affected cover ($p < 0.001$). In all GI2 and GP transects and for the GI1 mid-bed and deep transects cover was lower in 2007 compared to 2006. Cover at the shallow GI1 transect was significantly greater in 2007 compared to 2006 ($p < 0.001$).

Above Ground Biomass

On a regional level (across all sites) vegetative shoot above ground biomass was 4.2 times greater in 2007 than in 2006 (Table 4-2). Between sites in September and October 2006, above ground biomass decreased a second time at GP but did not decrease at the other two sites. This decline was followed by low biomass numbers in 2007 at this site while GI2 and GI1 biomass increased significantly ($p < 0.001$). As a result, biomass was 1.9 times greater at GI1 compared to GI2 ($p = 0.006$) and was 5.4 times greater than at GP ($p < 0.001$) resulting in the trend of increasing biomass (Figure 4-5). On the bed level (across all transects) biomass was 1.9 times greater in the near shore region of the bed compared to the deep edge ($p = 0.010$). However, there was no significant difference between the shallow edge and mid-bed regions ($p = 0.298$) or between the mid-bed and deep edge ($p = 0.109$).

While seedling biomass was similar to surviving vegetative shoot biomass in 2006, seedling biomass decreased to significantly lower levels in 2007 ($p < 0.001$). At GI1 seasonal means (April – October) in above ground biomass for seedlings decreased from 14.18 ± 2.07 g DW m⁻² in 2006 to 3.18 ± 2.20 g DW m⁻² in 2007 while surviving vegetative shoot biomass increased from 12.35 ± 1.69 g DW m⁻² to 54.96 ± 4.38 g DW m⁻². Similar trends were observed in both GI2 and GP sites (Figure 4-5).

No reproductive shoots were observed in 2006. In 2007 surviving vegetative plants produced a limited number of reproductive shoots; however, there were no observed reproductive shoots produced by seedlings (Figure 4-6). In addition, no reproductive shoots were observed at the GP site in 2007. Therefore reproductive shoot biomass was not analyzed for source or time and was only analyzed for the effects of site (GI1, GI2) and transect. GI1 had significantly greater reproductive shoot biomass compared to GI2 ($p < 0.001$). Seasonal (April – July) reproductive shoot biomass at GI1 averaged 18.46 ± 4.29 g DW m⁻² and 1.97 ± 0.61 g DW m⁻² at GI2. There was no significant difference in reproductive shoot biomass in GI1 or in GI2 regardless of transect position within the bed, (Table 4-2).

Density

As with biomass, regional vegetative shoot density was significantly greater in 2007 compared to 2006 ($p < 0.001$). GI1 had the greatest vegetative shoot density and produced 3.0 times more shoots than GI2 ($p = 0.001$) and 4.7 times more shoots than GP ($p < 0.001$; Table 4-3). There was no significant difference in density between GI2 and GP ($p = 0.187$). Shoot density did not differ on a site level and was not significantly different between the inner edge, mid-bed, or deep edge transects (Table 4-3). Seedling density significantly decreased from 48 – 54 % of total shoots in 2006 to only 0 – 6 % in 2007 a ($p < 0.001$). GI1 seedling density decreased from 410 ± 6 shoots m⁻² in 2006 to 52 ± 13 shoots m⁻² in 2007. In comparison vegetative shoot density increased from 376 ± 5 shoots m⁻² in 2006 to $1,063 \pm 60$ shoots m⁻² in 2007 at GI1. Similar trends were seen at the GI2 and GP with seedling shoot density decreasing from 207 ± 5 shoots m⁻² and 250

± 1 shoots m^{-2} to 4 ± 0 shoots m^{-2} and 5 ± 0 shoots m^{-2} . In addition, vegetative shoot density at GI2 and GP increased from 252 ± 6 shoots m^{-2} to 703 ± 32 shoots m^{-2} and from 84 ± 3 shoots m^{-2} to 260 ± 10 shoots m^{-2} respectively.

Reproductive shoot density was significantly affected by both site ($p = 0.003$) and position within the bed (Table 4-3). Compared across the flowering season, reproductive shoot density was 19.2 times greater at GI1 (133 ± 34 shoots m^{-2}) compared to GI2 (23 ± 6 shoots m^{-2}) and GP (0 ± 0 shoots m^{-2}). In addition reproductive shoots were 9.2 times denser at the deep edge of the bed compared to the shallow edge ($p = 0.026$) and 14.5 times denser than the mid-bed region ($p = 0.007$). There was no significant difference in reproductive shoot density between the mid-bed and shallow transects ($p = 0.644$). These trends are also reflected in the proportion of reproductive shoots produced at each site which was also significantly greater at GI1 ($p = 0.006$) and varied significantly across transects ($p = 0.005$; Table 4-4).

Seed-bank

The statistical model that best described the total seed density within the sediment seed-bank incorporated only the effects of date and transect (Table 4-5). Total sediment seed-bank density was not significantly affected by date ($p = 0.295$) or by region within the bed (Table 4-5). The distribution of seeds was patchy at all sites. Seed density ranged from 0 ± 0 seeds m^{-2} to 50 ± 24 seeds m^{-2} at GI1 in 2006 and from 0 ± 0 seeds m^{-2} to 91 ± 35 seeds m^{-2} in 2007 (Table 4-6). Seed-bank densities were lower in both GI2 and GP and ranged from 0 ± 0 to 25 ± 25 seeds m^{-2} and from 0 ± 0 to 33 ± 15 seeds m^{-2}

respectively. Of the seeds collected from the seed-bank in either site in 2006 and 2007, no seeds were found to be viable.

Discussion

The results of this study highlight the vulnerability of Chesapeake Bay *Z. marina* beds to repeated disturbance events once the loss of resiliency provided by a viable sediment seed-bank is removed. Following a sudden and large scale decline in the fall of 2005, *Z. marina* beds in the York River were re-established via both seedlings (48 – 54 % of total shoot density) and surviving vegetative shoots in < 1 year. By the summer of 2006 the populations had returned to pre 2005 decline population levels. During the second year of recovery in 2007 vegetative shoots were present at all beds while newly established seedlings contributed < 6 % of total shoot density. Throughout the recovery period few reproductive shoots were observed (2006: 0 % shoot density; 2007: < 1% of total shoot density) and no viable seeds were recorded in the sediment seed-bank leaving the seed-bank depleted. In the fall of 2006 the upriver bed (GP) suffered a second, consecutive decline presumably due to poor water quality conditions. The re-establishment at the upriver bed after the secondary decline was very limited compared to the recovery observed at the same site in 2006 ($1,020 \pm 485$ shoots m^{-2} in 2006; 168 ± 168 shoots m^{-2} in 2007). There was a lack of replenishment of the sediment seed-bank following the 2005 disturbance event that restricted capacity for bed re-growth at the upriver bed in 2007.

During the initial recovery stages (April – May 2006) seedlings dominated and contributed > 80% to total shoot density at all beds. By September 2006 total shoot

density shifted from seedling to vegetative shoot dominance and throughout 2007 < 6 % of shoots collected were seedlings. Similar trends of two step recruitment processes with initial re-colonization by seeds shifting to vegetative shoot dominance have been reported following large scale *Z. marina* die-backs (Plus et al., 2003; Greve et al., 2005; Lee et al., 2007). The shift to vegetative shoot dominance has been attributed to substantial (90-95 %) seedling mortality (Greve et al., 2005) and/or to increased rhizome elongation/branching over time in surviving vegetative shoots (Plus et al., 2003; Lee et al., 2007). The lack of a sudden decline in seedling density and biomass in this study indicates that a massive seedling mortality event did not occur in 2006 and that the shift is more likely due to greater rhizome branching rates by surviving vegetative shoots.

During the initial months of bed re-establishment following a sudden large scale decline seedlings have been found to grow at accelerated rates reaching a stable size by 3.5 months (Greve et al., 2005). As a result, seed production may only be limited and not completely lost for the first year post decline and return to or exceed pre-decline seed production levels after two years of growth (Lee et al., 2007). While seedlings in this study grew at an accelerated rate and reached maximum biomass numbers within 4 months, reproductive shoots were not observed during the first year post-decline and contributed <1 % to total shoot production during the second year of monitoring. For both years reproductive shoot densities were well below the 11-19 % Chesapeake Bay average (Silberhorn et al., 1983). In the Chesapeake Bay, seedlings typically have not been observed to flower until their second year of growth (Orth and Moore, 1986), therefore the lack of flowering in 2006 was not completely unexpected. Reduced flowering in 2007 may be attributed to interannual variation in flowering shoot

production suggesting that a variety of factors can affect *Z. marina* flowering success (Orth and Moore, 1986).

As a result of reduced flowering, seed production was limited and the sediment seed-bank was not replenished in 2006. *Z. marina* seed-banks are transient and seeds remain viable for < 1 year once incorporated into the sediment (Lamounette, 1977; Orth et al., 2000; Jarvis Chapter 2). The large scale germination event following the 2005 decline depleted the sediment seed-bank in the lower York River from an average of 605 ± 275 seeds m^{-2} (Harwell and Orth, 2002) to a maximum density of 50 ± 24 seeds m^{-2} . When viability is taken into account, seed-bank density decreased from 330 ± 330 seeds m^{-2} (Harwell and Orth, 2002) to 0 ± 0 seeds m^{-2} . The lack of replenishment in both 2006 and 2007 resulted in the loss of the ecological function of the seed-bank for a minimum of two years following the decline event.

The impact of the loss of resiliency provided by the sediment seed-bank on Chesapeake Bay *Z. marina* beds was highlighted after a secondary decline occurred in the upriver site in 2006. The upriver site declined presumably after low light conditions (< 22 % of available light) coincided with high summer water temperatures (> 25 °C). While water temperatures did not increase above the 30 °C threshold lethal to adult *Z. marina* plants, water temperatures above 25 °C are stressful and can result in reduced production and survival (Nerjup and Pederson, 2008). The effect of high water temperatures on seedlings is unknown. Under laboratory conditions, low light conditions (< 23 % of incident light) resulted in reduced lateral shoot production and seedling biomass (Bintz and Nixon, 2001). Therefore, in areas where seedlings receive < 23 % of incident light during their first summer of growth long-term survival is unlikely, since a

reduction in light levels impedes clonal reproduction. During the 2006 growing season, light levels were reduced below 22 % of incident light from June to October in the upriver site. The combined effects of low light and high temperature conditions may have had a significant impact on seedling survival resulting in the secondary decline. In 2007, no seedlings were observed, vegetative shoot density did not increase above 21 shoots m⁻², and biomass did not increase above 5.26 g m⁻² resulting in a nearly complete loss of *Z. marina* at this site.

The limited re-establishment of *Z. marina* at the upriver site in 2007 highlights both the important role of seedlings in the initial phase following a large scale decline and the vulnerability of *Z. marina* beds to repeated disturbance. Protected in the sediment seed-bank, *Z. marina* seeds were able to survive the high summer water temperatures in July and August 2005 and germinate in the fall when water temperatures returned to tolerable levels. A large scale germination event following the 2005 decline initiated the recovery at all sites in 2006; however, it also depleted the sediment seed-bank. Reduced flowering in the spring of 2006 combined with a lack of viability of the remaining seeds in the sediment seed-bank resulted in a reduced seed-bank for at least one year following the 2005 large scale germination event. Therefore, following a second decline in the summer of 2006 the recovery of the upriver site was limited by a lack of seed germination and seedling establishment.

Adaptations for *Z. marina* beds to repeated disturbances in the Chesapeake Bay are limited. While sexual reproduction in *Z. marina* beds can increase in response to disturbances (Phillips et al., 1983; van Lent and Verschuure, 1994), flowering in perennial *Z. marina* seedlings is limited and the effects of stress on *Z. marina* flowering

are unknown. Although annual and semi-annual *Z. marina* seedlings are capable of flowering, the development of annual and semi-annual beds is poorly understood (Keddy and Patriquin, 1978; Meling-Lopez and Ibarra-Obando, 1999; Jarvis Chapter 1) and to date no annual or semi-annual populations have been observed in the Chesapeake Bay. During the next several decades, perturbations due to increased water temperatures (Preston, 2004) and low light conditions as a result of rising sea level and climate changes (Harley et al., 2006) are predicted to increase in coastal systems. Based on the results shown here, without adaptation, multiple consecutive years of disturbance related declines may result in large scale, long-term loss of *Z. marina* in the Chesapeake Bay.

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Table 4-1. Negative binomial results and odds ratios for the effects of year, site, and their interactions on *Z. marina* shoot density in the lower York River, VA between 2004 – 2007. All significant results are denoted with an (*).

Parameter	DF	Est	SE	X ²	p	odds ratio	Wald 95 % CL	
							Low	High
Intercept	1	5.59	0.15	1319.27	<0.001*	--	--	--
Site								
GI 1	0	--	--	--	--	--	--	--
GI 2	1	-0.21	0.31	0.46	0.495	0.81	0.44	1.48
GP	1	0.12	0.37	0.11	0.744	1.13	0.54	2.34
Date								
2004	1	--	--	--	--	--	--	--
2005	1	-0.68	0.24	8.25	0.004*	0.51	0.32	0.81
2006	1	-0.57	0.19	9.10	0.003*	0.57	0.39	0.82
2007	1	-0.73	0.19	14.23	0.000*	0.48	0.33	0.71
Site*Date								
GI1*2004	0	--	--	--	--	--	--	--
GI1*2005	0	--	--	--	--	--	--	--
GI1*2006	0	--	--	--	--	--	--	--
GI1*2007	0	--	--	--	--	--	--	--
GI2*2004	1	--	--	--	--	--	--	--
GI2*2005	1	0.49	0.46	1.14	0.285	1.64	0.66	4.02
GI2*2006	1	0.28	0.38	0.55	0.456	1.32	0.63	2.76
GI2*2007	1	-0.11	0.38	0.08	0.778	0.90	0.42	1.90
GP*2004	1	--	--	--	--	--	--	--
GP*2005	1	-0.39	0.55	0.51	0.475	0.67	0.23	1.99
GP*2006	1	-0.77	0.46	2.51	0.094	0.46	0.19	1.14
GP*2007	1	-2.64	0.47	31.53	<0.001*	0.07	0.03	0.18
Dispersion	1	3.22	0.13					

Table 4-2. Negative binomial results and odds ratios for the effects of year, and site on *Z. marina* vegetative above ground biomass. Reproductive shoot biomass was analyzed for the effects of site and transect only. All significant results are denoted with an (*).

							Wald 95 % CL	
Parameter	DF	Est	SE	X ²	p	odds ratio	Low	High
<u>Vegetative Shoots</u>								
Intercept	1	-2871.80	389.64					
Time	1	1.43	0.19	54.44	<0.001*	4.19	2.86	6.13
Transect								
Shallow	0	--	--	--	--	--	--	--
Mid-Bed	1	0.25	0.24	1.08	0.298	1.20	0.80	2.07
Deep	1	0.63	0.25	6.69	0.010*	1.88	1.17	3.04
Site								
GI1	0	--	--	--	--	--	--	--
GI2	1	-0.65	0.24	7.66	0.006*	0.52	0.33	0.83
GP	1	-1.69	0.25	47.33	<0.001*	0.19	0.11	0.30
Source	1	8276.25	606.83	186.01	<0.001*	--	--	--
Time*Source	1	-4.12	0.30	186.08	<0.001*	0.02	0.11	0.30
Transect*Source								
Shallow*Source	0	--	--	--	--	--	--	--
Mid-Bed*Source	1	-0.53	0.35	2.25	0.133	0.59	0.30	1.18
Deep*Source	1	-0.85	0.36	5.53	0.019*	0.43	0.21	0.87
Site*Source								
GI1*Source	0	--	--	--	--	--	--	--
GI2*Source	1	-1.38	0.36	14.27	<0.001*	0.25	0.12	0.52
GP*Source	1	-0.12	0.37	0.09	0.758	0.90	0.43	1.85
Dispersion	1	5.71	0.32					
<u>Reproductive Shoots</u>								
Intercept	1	2.53	0.51					
Site	1	-1.60	0.53	8.93	<0.001*	0.20	0.07	0.58
Transect								
Shallow	0	0.00	0.00	0.00	--	--	--	--
Mid-bed	1	-0.73	0.64	1.31	0.252	0.48	0.14	1.68
Deep	1	0.49	0.63	0.61	0.435	1.63	0.48	5.55
Dispersion	1	9.43	1.62					

Table 4-3. Negative binomial results and odds ratios for the effects of year, and site on vegetative *Z. marina* shoot density. Reproductive shoots were analyzed for site and transect only. All significant results are denoted with an (*).

Parameter	DF	Est	SE	X ²	P	odds ratio	Wald 95 % CL	
							Low	High
<u>Vegetative Shoots</u>								
Intercept	1	-2059.83	552.632	13.89	<0.001*			
Date	1	1.03	0.275	13.95	<0.001*	2.80	1.63	4.80
Transect								
Shallow	0	0.00	0.000	0.00	--	--	--	--
Mid-bed	1	0.35	0.341	1.05	0.305	1.42	0.73	3.42
Deep	1	0.56	0.342	2.69	0.101	1.75	0.90	9.23
Site								
GI1	0	0.00	0.00	0.00	--	--	--	--
GI2	1	1.10	0.34	10.50	0.001*	3.00	1.54	5.84
GP	1	1.55	0.34	20.46	<0.001*	4.69	2.40	9.16
Source	1	8760.32	820.11	114.10	<0.001*	--	--	--
Date*Source	1	-4.37	0.41	114.12	<0.001*	0.01	0.01	0.03
Source*Transect								
Shallow	0	0.00	0.00	0.00	--	--	--	--
Mid-bed	1	-0.18	0.48	0.14	0.713	0.84	0.33	2.14
Deep	1	-0.26	0.50	0.27	0.603	0.77	0.29	2.05
Source*Site								
GI1	0	0.00	0.00	0.00	--	--	--	--
GI2	1	-1.19	0.50	5.74	0.017*	0.30	0.11	0.80
GP	1	0.11	0.52	0.05	0.827	1.12	0.41	3.08
Dispersion	1	11.77	0.58	--	--	--	--	--
<u>Reproductive Shoots</u>								
Intercept	1	5.05	0.71	51.97	<0.001*			
Site	1	-2.99	0.99	9.02	0.003*	0.05	0.01	0.35
Transect								
Shallow	0	0.00	0.00	0.00	--	--	--	--
Mid-bed	1	-2.21	0.99	4.98	0.026*	0.11	0.02	0.76
Deep	1	0.46	0.99	0.21	0.644	1.58	0.23	11.03
Site*Transect								
Site*Shallow	0	0.00	0.00	0.00	--	--	--	--
Site*Mid-bed	1	3.19	1.41	5.16	0.023*	24.39	1.55	383.41
Site*Deep	1	1.15	1.40	0.67	0.413	3.16	0.20	49.55
Dispersion	1	9.82	1.63					

Table 4-4. Mean percent of total *Z. marina* flowering shoots found in the shallow, mid-bed, and deep regions of the GI1, GI2, and GP sites in 2006 and 2007. Values mean \pm S.E.

Date	GI1			GI2			GP		
	Shallow	Mid-Bed	Deep	Shallow	Mid-Bed	Deep	Shallow	Mid-Bed	Deep
2006									
April	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
May	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
June	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
July	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
2007									
April	4 \pm 3	2 \pm 2	41 \pm 15	3 \pm 3	0 \pm 0	20 \pm 9	0 \pm 0	0 \pm 0	0 \pm 0
May	36 \pm 17	7 \pm 6	19 \pm 1	1 \pm 1	4 \pm 3	4 \pm 2	0 \pm 0	0 \pm 0	0 \pm 0
June	0 \pm 0	0 \pm 0	3 \pm 2	0 \pm 0	7 \pm 7	1 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0
July	11 \pm 6	0 \pm 0	3 \pm 2	0 \pm 0	1 \pm 1	1 \pm 1	0 \pm 0	0 \pm 0	0 \pm 0

Table 4-5. Negative binomial results and odds ratios for the effects of date and transect on total *Z. marina* seed-bank density.

Parameter	DF	Est	SE	X ²	p	odds ratio	Wald 95 % CL	
							Low	High
Intercept	1	-1217.58	1166.60	1.09	0.297			
Date	1	0.61	0.58	1.10	0.295	1.84	0.59	5.74
Transect								
Shallow	0	0.00	0.00	0.00	--	--	--	--
Mid-bed	1	-0.43	0.69	0.38	0.537	0.65	0.17	2.53
Deep	1	-0.81	0.68	1.42	0.233	0.44	0.12	1.69
Dispersion	1	47.82	5.85					

Table 4-6. Mean seed-bank density and seed viability in *Z. marina* seeds collected from the ambient sediment seed-bank at GI1, GI2, and GP sites in 2006 and 2007.

	Total Seed-bank Density			% Viable Seeds		
	GI1	GI2	GP	GI1	GI2	GP
2006						
April	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
May	0 ± 0	33 ± 19	25 ± 13	0 ± 0	0 ± 0	0 ± 0
June	17 ± 11	33 ± 19	0 ± 0	0 ± 0	0 ± 0	0 ± 0
July	8 ± 8	8 ± 8	8 ± 8	0 ± 0	0 ± 0	0 ± 0
Aug	50 ± 24	41 ± 29	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sept	33 ± 19	0 ± 0	25 ± 18	0 ± 0	0 ± 0	0 ± 0
Oct	0 ± 0	0 ± 0	33 ± 15	0 ± 0	0 ± 0	0 ± 0
2007						
April	8 ± 8	8 ± 8	8 ± 8	0 ± 0	0 ± 0	0 ± 0
May	50 ± 24	8 ± 8	25 ± 13	0 ± 0	0 ± 0	0 ± 0
June	83 ± 23	17 ± 11	33 ± 15	0 ± 0	0 ± 0	0 ± 0
July	33 ± 15	25 ± 25	8 ± 8	0 ± 0	0 ± 0	0 ± 0
Aug	50 ± 20	17 ± 11	8 ± 8	0 ± 0	0 ± 0	0 ± 0
Sept	91 ± 35	0 ± 0	17 ± 11	0 ± 0	0 ± 0	0 ± 0
Oct	17 ± 11	25 ± 18	8 ± 8	0 ± 0	0 ± 0	0 ± 0

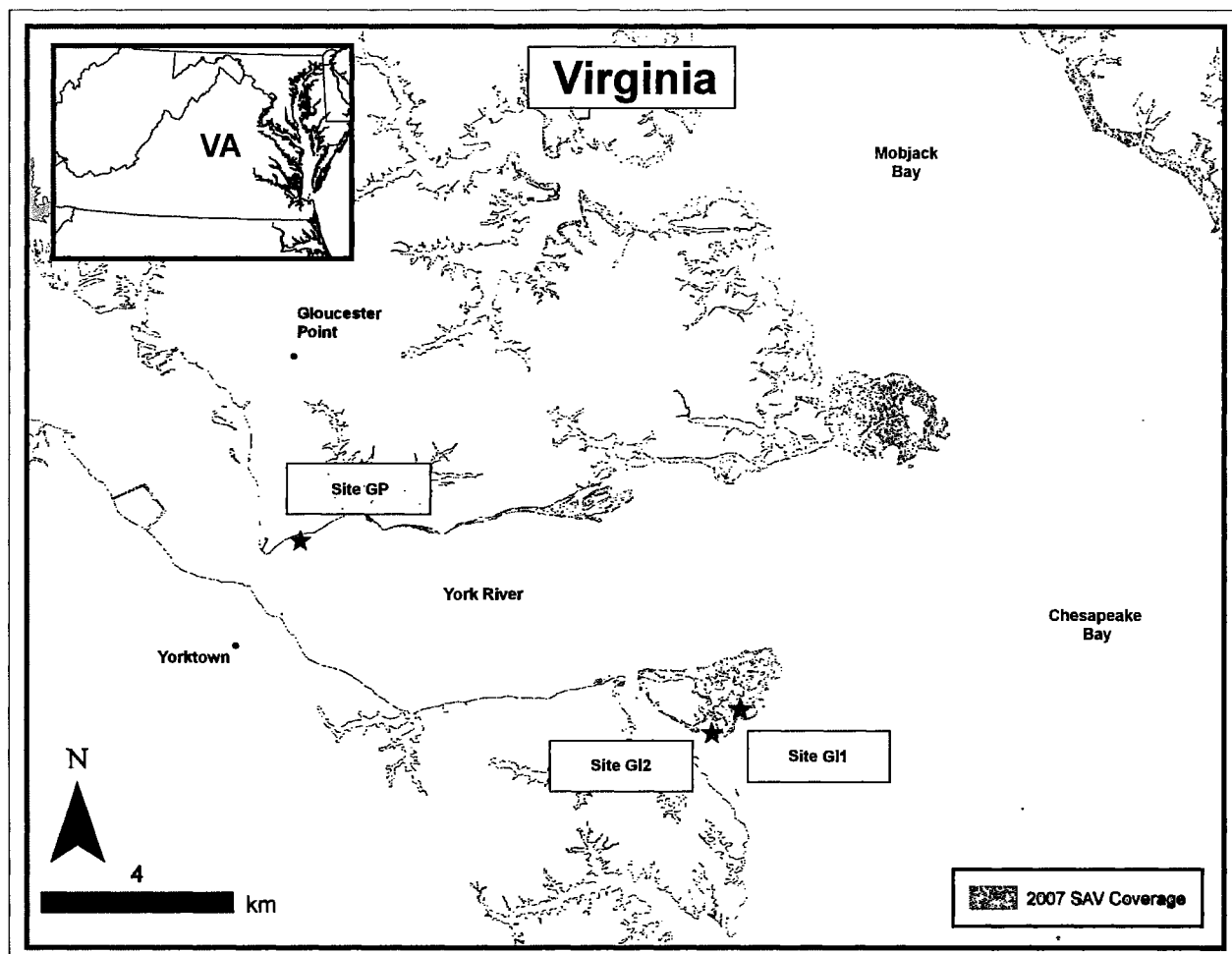


Figure 4-1. Study site location with respect to the Chesapeake Bay. GI1 and GI2, the down river sites, are located at the mouth of the York River and GP, the upriver site, was located 10 km up river at Gloucester Point. Site locations are denoted with a star.

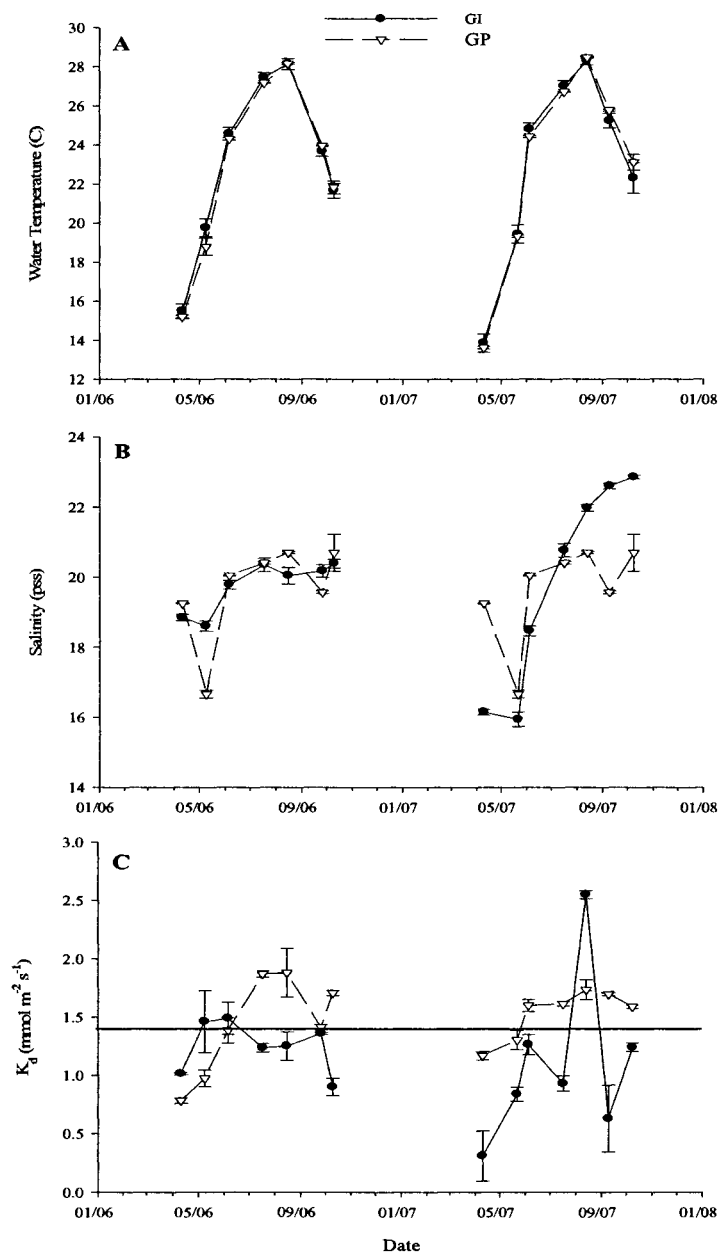


Figure 4-2. Water quality conditions at both the up river (GP) and down river sites (GI). Monthly mean (A) water temperature, (B) salinity, (C) and light attenuation values are given for 2006 and 2007. Values are \pm S.E. The black line represents 22 % light available through the water column.

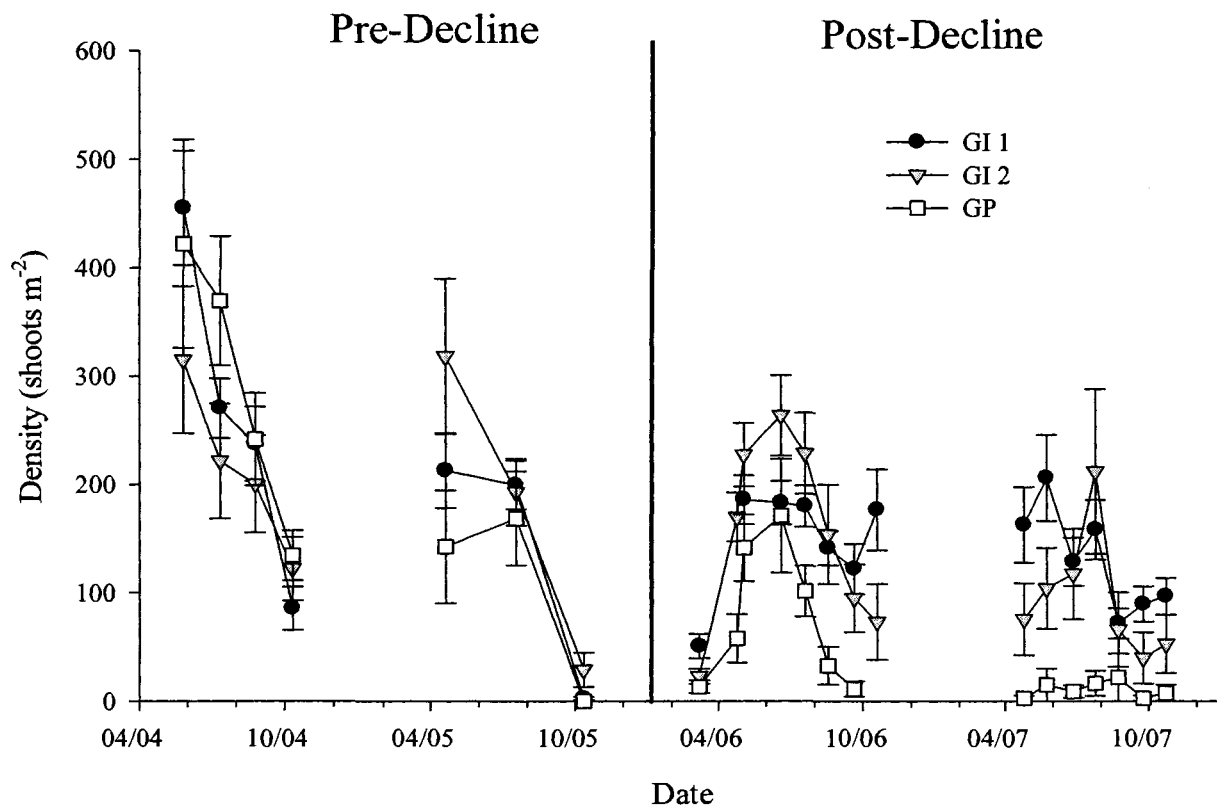


Figure 4-3. *Z. marina* density at three locations in the lower York River, VA from 2004 – 2007. Black line marks large scale decline of *Z. marina* in the lower Chesapeake Bay in 2005. Mean density \pm standard error values are given for the up river (GI1 – black circle, GI2 - grey triangle and down river (GP – white square) sites for both pre-decline and post-decline time periods.

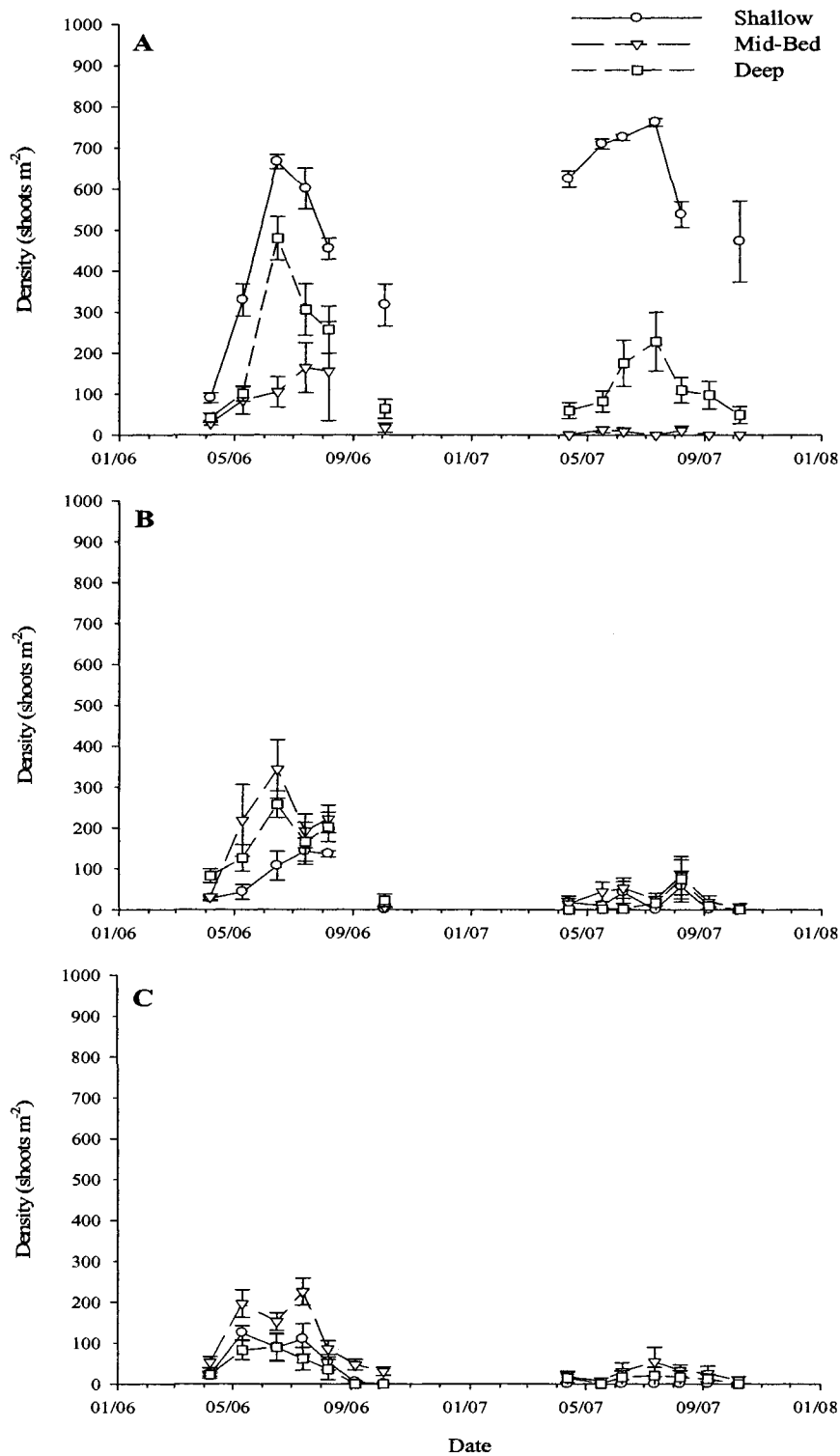


Figure 4-4. Mean *Z. marina* density for the down river (A – GI1, B – GI2), and upriver (C – GP) shallow, medium, and deep transects in 2006 – 2007. Values are reported as mean \pm standard error.

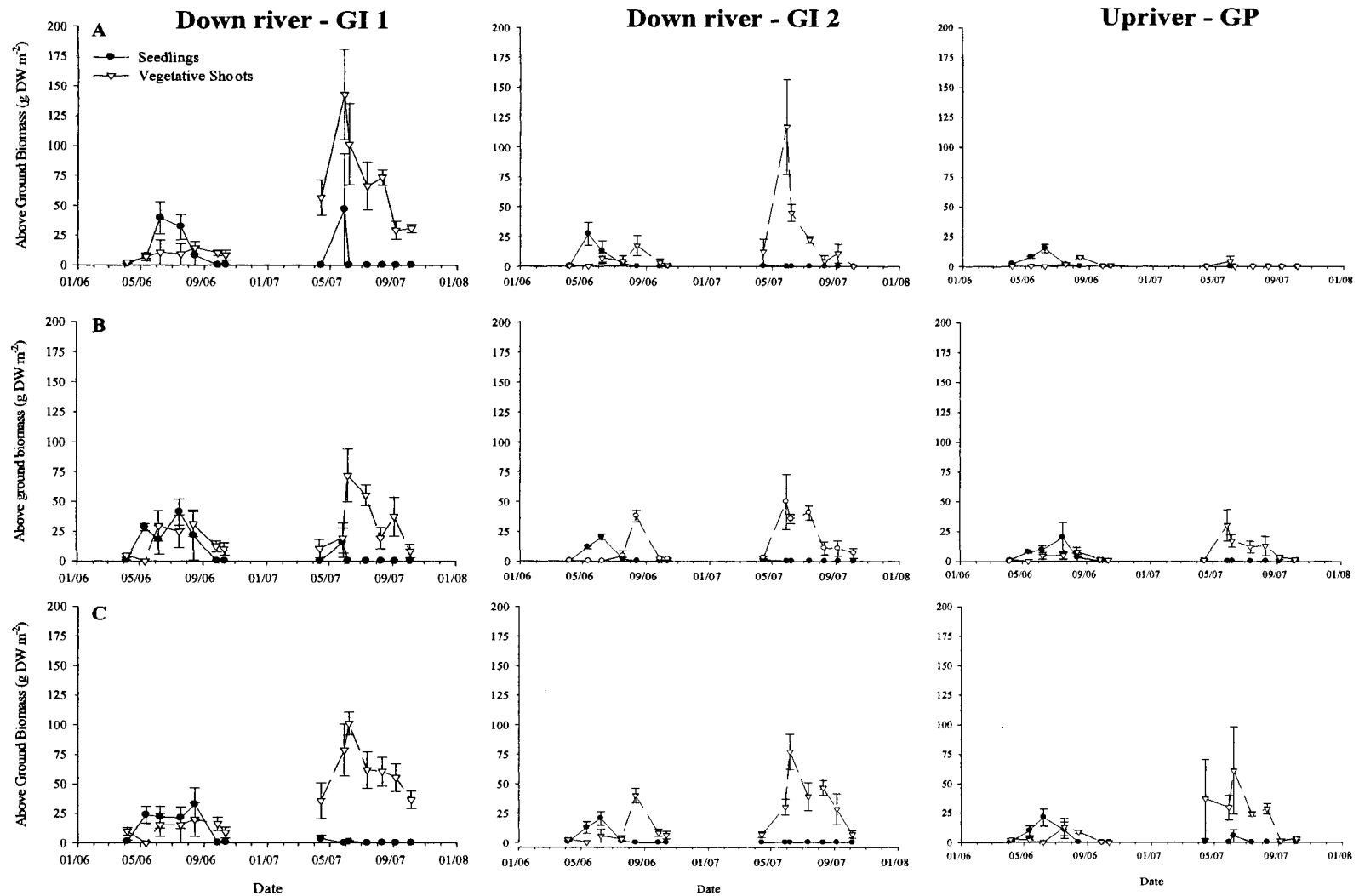


Figure 4-5. Mean vegetative shoot above ground biomass for all sites. Data are shown for the (A) shallow (B) mid-bed and (C) deep transects. Values are shown as mean \pm standard error.

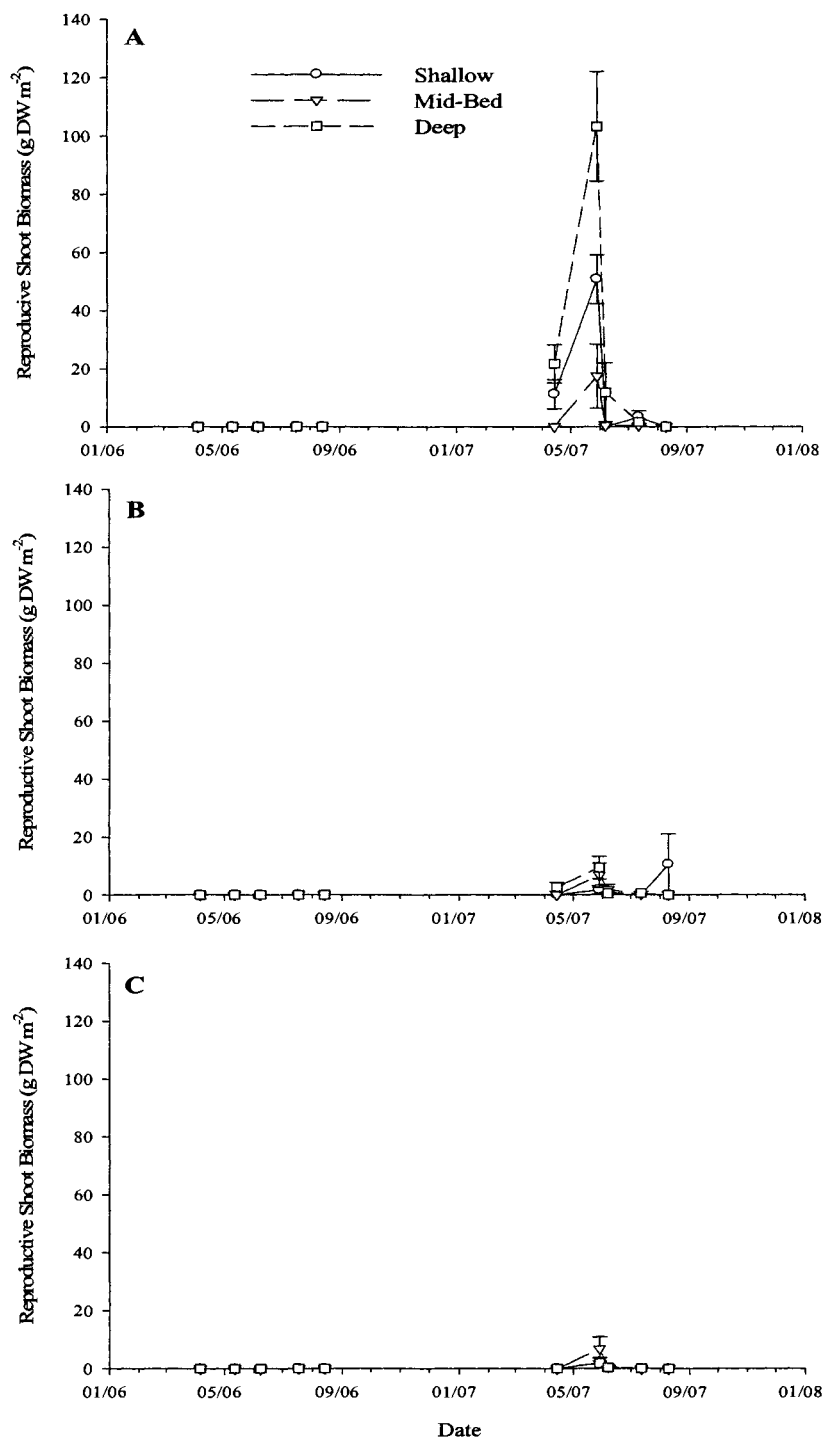


Figure 4-6. Mean reproductive shoot above ground biomass at the down river (A – GI1, B – GI2), and upriver (C – GP) sites. Values are shown as mean \pm standard error.

CHAPTER 5 : MODELING LOSS AND RECOVERY OF ZOSTERA MARINA BEDS
IN THE CHESAPEAKE BAY FOLLOWING REPEATED DISTURBANCE EVENTS:
THE ROLE OF SEEDLINGS AND SEED-BANK VIABILITY.

Abstract

The loss and recovery processes following a large scale decline in *Zostera marina* in the York River, Virginia was modeled using a *Z. marina* production model containing a sexual reproduction component. Reproductive shoot production, total seeds produced, density of seeds in the seed-bank, seed viability, and seed germination were added to determine the contribution of sexual reproduction to bed resilience. The base model was calibrated to *Z. marina*, water quality, and sediment data collected from a *Z. marina* bed at Goodwin Island located at the mouth of the York River and validated using data from a bed located 10 km up river at Gloucester Point. For both sites, model scenarios were run for three years (2005 – 2007) where the effects of (1) the presence or absence of sexual reproduction (2) projected increases in water temperature from ambient to ambient +5 °C in 1 °C increments; and (3) the potential interactive effects of low light and high temperature conditions on bed maintenance and re-establishment were quantified. Model projections of *Z. marina* production following the 2005 decline and subsequent recovery period were more accurate relative to *in situ* measurements when sexual reproduction was added compared to the traditional production model with vegetative reproduction only. Following the 2005 decline, in simulations where sexual reproduction was included, model results project an initial period of recovery in 2006 in all temperature treatments. Without the inclusion of sexual reproduction, there is no projected recovery following the 2005 decline regardless of temperature. However, resiliency to increased water temperature provided by sexual reproduction was limited, as a 1 °C increase in temperature resulted in a reduction of biomass to 0 g C m⁻² by year three. Differences in water quality between sites also affected *Z. marina* above and below ground production,

seed production, seed viability and seed germination. The combination of lower light and temperature stress present at the upriver site resulted in overall lower production and resiliency to declines compared to the down river site. The results of all model scenarios highlight the need to incorporate sexual reproduction into *Z. marina* ecosystem models, the projected sensitivity of established *Z. marina* beds to consecutive years of stress, and the negative effects of multiple stressors on *Z. marina* recovery.

Key Words: *Zostera marina*, sexual reproduction, ecological model, seeds

Introduction

Seagrass populations, vital components of coastal ecosystems, have declined globally over the last several decades (den Hartog, 1970; Orth et al., 2006; Short et al., 2006). Global declines, predicted to increase over time (Duarte, 2002), have been linked to coastal development (Short and Wyllie-Echeverria, 1996), eutrophication (Short et al., 2006a), and climate change (Short and Neckles, 1999). Within the Chesapeake Bay, Virginia large scale declines in *Zostera marina* populations have been attributed to chronic declines in water quality compounded by episodic stresses from short term events such as tropical storms or high water temperatures (Orth and Moore, 1983; Moore and Jarvis, 2008). Restoration attempts in the Chesapeake have increased in response to continued declines; however, efforts have proven to be primarily unsuccessful (Shafer and Bergstrom, 2008). In order to increase restoration efficiency, effectiveness, and success a better understanding of bed resiliency to perturbations, as well as loss and recovery processes within established seagrass beds is required (Duarte, 2002; Orth et al., 2006).

Ecological models are a useful tool in quantitative analysis of complex ecosystems such as seagrass beds. Through models, the response of *Z. marina* to stressful environmental conditions such as low light, high nutrients, and high temperatures has been quantified under a variety of situations (Wetzel and Neckles, 1986; Bach, 1993; Aveytua-Alcázar et al., 2008). While these models provide insight into the singular and combined effects of environmental stressors on *Z. marina* production, the capacity to accurately model population responses to stressful conditions is limited by focusing solely on vegetative reproduction and ignoring sexual reproduction (van Lent,

1995). Exclusion of sexual reproduction in carbon based models has been accepted due to the dominance of vegetative reproduction in perennial *Z. marina* beds and the relatively low carbon value of seeds (Harwell, 2000). However, recent research has shown that sexual reproduction plays a significant role in *Z. marina* bed recovery from large scale declines (Plus et al., 2003; Greve et al., 2005); therefore a key component of the bed loss and recovery dynamic may be missing from *Z. marina* production models when sexual reproduction is excluded.

Sexual reproduction and seed production in *Z. marina* beds has been observed to increase under stressful environmental conditions such as extreme temperatures (Phillips et al., 1983). For perennial *Z. marina* populations, seeds in the sediment seed-bank provide a measure of resiliency to large scale loss (Leck et al., 1989; Combroux, 2001). Recolonization of *Z. marina* beds following sudden large scale declines through seed germination and seedling establishment have been documented throughout the species range (Plus et al., 2003; Greve et al., 2005; Lee et al., 2007). However, for the seed-bank to provide any function, seed viability must be maintained (Leck et al., 1989). Perennial *Z. marina* beds in the Chesapeake Bay region produce transient seed-banks (seeds remain viable in the sediment for < 1 year) and must be replenished annually (Orth et al., 2000; Jarvis Chapter 3). Successful germination of viable seeds is dependent upon environmental cues and the surrounding sediment microenvironment (Moore et al., 1993; Probert and Brenchly, 1999). Ecological models need to consider seed production, seed-bank density, seed viability, and germination to accurately incorporate sexual reproduction into seagrass production models.

Although vegetative reproduction is dominate in most beds, sexual reproduction may become more important in Chesapeake Bay *Z. marina* beds as habitat conditions are predicted to become increasingly stressful (Najjar, 1999; Gibson and Najjar, 2000). Over the last fifty years (1949 – 2002) Chesapeake Bay winter water temperatures have increased 0.8 – 1.1 °C and are predicted to continue to increase over the next several decades (Preston, 2004). *Z. marina* is a temperate seagrass species (den Hartog, 1970), and an increase of 1 °C may have a significant impact on Chesapeake Bay *Z. marina* beds as these populations are located near the southern limit of the species distribution along the western Atlantic where high summer water temperatures are limiting (Short and Moore, 2006). In addition, disturbances in the form of more frequent large scale storms and increases in rainfall are predicted to occur over the next century as one result of global climate change (Harley, 2006). Climate change models predict that a 10 % increase in rainfall over the Chesapeake Bay watershed will subsequently increase river flow by 30 %, thereby increasing the nutrient and sediment input into the bay (Najjar, 1999; Gibson and Najjar, 2000). As a result of increased nutrient and sediment loads the amount of light available to *Z. marina* will likely decrease, further stressing *Z. marina* populations (Dennison et al., 1993).

Large scale declines in *Z. marina* populations due to increased water temperatures have already been observed in the Chesapeake Bay (Moore and Jarvis, 2008). In the fall of 2005 a sudden and large scale decline of *Z. marina* occurred in the lower York River, a tributary to the Chesapeake Bay (Moore and Jarvis, 2008). The loss of above ground biomass occurred after an extended period of summer water temperatures > 27 °C. By 2007 *Z. marina* beds were re-established in the mouth of the river; however 10 km

upriver *Z. marina* re-establishment was limited. The objective of this study was to gain a better understanding of the role of sexual reproduction in established perennial *Z. marina* beds subject to temperature and light stress relative to a large scale decline through modeling the 2005 *Z. marina* decline and a two year re-establishment period in the lower York River. Sexual reproduction and a seed sub-model were used in a *Z. marina* productivity model that was developed based on the established *Z. marina* models of Madden and Kemp (1996), Buzzelli et al. (1999), and Cerco and Moore (2001). The effects of (1) the presence or absence of sexual reproduction, (2) projected increases in water temperature from ambient to ambient +5 °C, and (3) the potential interactive effects of low light and high temperature conditions on bed maintenance and re-establishment in the model were simulated over the three year period. Specifically we quantified percent change between ambient (base model conditions) and model scenarios in *Z. marina* above and below ground biomass, total seed production, seed-bank density, and seed germination.

Methods

Model Description

An established perennial *Z. marina* bed in the lower York River was chosen as a basis for the production and reproduction models due to the documented decline of *Z. marina* shoots in this area in the fall of 2005 (Moore and Jarvis, 2008). The model was calibrated using data from a site located at the mouth of the York River at the Goodwin Island National Estuarine Research Reserve (GI; 37° 13' N; 76° 23' W; Figure 5-1). Once calibrated, the model was validated using data from a site located 10 km up river adjacent

to the Virginia Institute of Marine Science Gloucester Point campus (GP; 37° 14.8' N, 76° 30.3' W; Figure 5-1). Both sites have been monitored since 2004 as part of the Chesapeake Bay National Estuarine Research Reserve Tier II Biological Monitoring Program for interannual variation in cover and density.

The base *Z. marina* productivity model was modified from Madden and Kemp (1996), Buzzelli et al. (1999), and Cerco and Moore (2001). State variables included epiphyte biomass (C_{epi}), *Z. marina* vegetative shoot biomass (C_{zms}), *Z. marina* vegetative root biomass (C_{zmr}), *Z. marina* seed-bank density (Z_{mseed}); *Z. marina* seedling density (Z_{msd}); *Z. marina* seedling shoot biomass (C_{zmss}), and *Z. marina* seedling root biomass (C_{zmsr} ; Figure 2). Biomass was quantified as g C m^{-2} and density was quantified as seeds or shoots m^{-2} . Forcing functions included water temperature ($^{\circ}\text{C}$), photoperiod (F), photosynthetically active radiation (PAR, $\mu\text{E m}^{-2} \text{s}^{-1}$), water column chlorophyll *a* ($\mu\text{g l}^{-1}$), total suspended solids (mg l^{-1}), water column and sediment dissolved inorganic nitrogen ($\text{DIN} - \text{NO}_x + \text{NH}_4$, $\mu\text{mol l}^{-1}$), water column and sediment dissolved inorganic phosphorus ($\text{DIP} - \text{PO}_4^{-3}$, $\mu\text{mol l}^{-1}$), sediment organic content (% organic), and seed burial depth (cm; Figures 5-3 and 5-4).

The initial model was developed with a simulation time of 1.5 years (April 1, 2006 through December 31 2007) with a time step (dt) of 0.125 days. The baseline model was developed to reproduce conditions at a water depth of 0.5 m in established *Z. marina* beds in the York River. Calibration values for above and below ground biomass, flowering shoot production, and seed densities were derived from monitoring data, field experiments, and literature values (Buzzelli et al., 1999; Harwell, 2000; Cerco and Moore 2001; Harwell and Orth 2002; Nerjup et al., 2008). Data for forcing functions were

collected from the Virginia Institute of Marine Science long term bi-weekly shallow water monitoring program (Moore unpublished data) and from the Chesapeake Bay National Estuarine Research Reserve of Virginia (CBNERRVA) water quality and meteorological monitoring stations located at the Goodwin Islands and Taskinas Creek sites respectively (<http://www2.vims.edu/vecos/>).

Z. marina production utilized both water column and sediment nutrients while epiphytes used water column nutrients only. PAR available to epiphytes was modified by water column chlorophyll a and total suspended solids while PAR available to *Z. marina* was also attenuated by epiphyte biomass. *Z. marina* biomass was converted to shoot density and a fraction of the total shoot density flowered and produced seeds. After seed germination carbon was returned to the production model through seedling above and below ground biomass (Figure 5-2).

Model Formulation

Production Model

Governing equations for *Z. marina* vegetative and seedling shoot biomass were balanced between gains through photosynthesis and losses due to mortality, respiration and translocation to roots and rhizomes (Table 5-1). Epiphytes were balanced similarly with the added loss of grazing but no loss due to translocation. Production terms for both epiphytes and *Z. marina* shoots were computed as the product of a temperature dependent maximum rate (P_{max}) and a limiting factor:

$$PR = P_{max} * MIN[PAR, DIN, DIP] \quad (1)$$

in which either nutrients (DIN, DIP) or light (PAR) were limiting (Madden and Kemp, 1996; Cerco and Moore, 2001). Maximum epiphyte production rates for GI were taken from Buzzelli et al. (1999; Eq 2) and production rates for *Z. marina* were determined from Evans et al. (1986; Eq 3):

$$P \max_{epi} = 0.003 * T_w * \left[1.0 - \frac{(T_w - 25)}{20} \right] \quad (2)$$

$$P \max_{zm} = 0.0948 + 0.0309 * e^{\left(-0.5 * \left(\frac{(T_w - T_{opt})}{3.2964} \right)^2 \right)} \quad (3)$$

In both cases maximum production is related to ambient water temperatures (T_w) and optimum water temperatures (T_{opt} ; Table 5-2).

Z. marina shoot and epiphyte production were limited by available light and nutrient concentrations. PAR was calculated similarly to Madden and Kemp (1996) where forced incident light was reduced in successive stages. Initial light availability was reduced exponentially to depth (z) with a Beer Lambert equation (Kirk, 1983):

$$PAR_2 = PAR_1 * e^{(-K_d * z)} \quad (4)$$

Down-welling light attenuation coefficient (K_d) accounted for additive effects of chlorophyll a , TSS, and the water itself on light availability in the water column:

$$K_d = (0.054 * Chl^{0.667} + 0.0088 * Chl) + (0.0396 * TSS + 0.39) + 0.03 \quad (5)$$

(Madden and Kemp, 1996). Total light available to *Z. marina* leaves (PAR_1) was further attenuated by a simulated epiphyte layer based on the units of epiphyte biomass located on the leaf blade:

$$PAR_3 = PAR_2 * e^{(0.32 - (0.42 * 2.5 * C_{epi}))} \quad (6)$$

(Madden and Kemp, 1996). For both epiphytes and *Z. marina* light limitation on P_{\max} values was calculated using Michaelis-Menteon kinetics:

$$PAR = \frac{PAR}{(K_{PAR} + PAR)} \quad (7)$$

where light availability (PAR) is limited by a light half saturation constant (K_{PAR} Table 5-2; Madden and Kemp, 1996).

Nutrient limitation in epiphytes was computed similarly to light limitation with PAR substituted with water column nutrient concentrations (N_w) and the light half saturation constant with nutrient half saturation constants for epiphytes (Table 5-2). For *Z. marina* multiple sources of nutrients (sediment and water column) were taken into account with a Monod-like function for nutrient limitation:

$$f(N) = \frac{(N_w + K^* * N_s)}{(K_{hw} + N_w + K^* * N_s)} \quad (8)$$

where N_w = water column nutrient concentrations ($\mu\text{mol l}^{-1}$) in the water column, N_s = sediment nutrient concentrations ($\mu\text{mol l}^{-1}$); and K_{hw} = half saturation constant for nutrient uptake ($\mu\text{mol l}^{-1}$) by shoots (Madden and Kemp, 1999; Cerco and Moore, 2001). In addition:

$$K^* = \frac{K_{hw}}{K_{hs}} \quad (9)$$

where K_{hs} = half saturation constant for nutrient uptake ($\mu\text{mol m}^{-3}$) by roots (Madden and Kemp, 1999; Cerco and Moore, 2001).

Losses from epiphytes and *Z. marina* shoots were attributed to mortality (leaf sloughing in *Z. marina*) and respiration (Madden and Kemp, 1996; Buzzelli et al., 1999). *Z. marina* had an additional loss term of translocation to the roots while epiphyte

production was also lost to grazing (Buzzelli et al., 1999). Epiphyte mortality was a function of a density dependent mortality constant and the ratio of epiphyte and *Z. marina* shoot carbon:

$$M_{epi} = MR_{epi} * \frac{C_{epi}}{C_{zm}} \quad (10)$$

where MR_{epi} = the epiphyte mortality constant (Table 2; Buzzelli et al., 1999). *Z. marina* mortality was a combination of a constant *Z. marina* mortality term over time and a temperature dependent function:

$$M_{zms} = MR_{zms} + 0.0175 - 0.125 * \cos\left(\frac{2.0 * \pi * JD}{365}\right) * \left(\frac{(T_w - 23)}{10}\right) * C_{zms} \quad (11)$$

where MR_{zms} = constant mortality rate over time (day^{-1} , Table 5-2) and JD = Julian Day (Buzzelli et al., 1999).

Epiphyte respiration was temperature dependent where KtB_{epi} = constant epiphyte respiration rate and BMR_{epi} = epiphyte basal respiration rate:

$$R_{epi} = C_{zms} * BMR_{epi} * e^{(KtB_{epi} * (T_w - T_{opt}))} \quad (12)$$

(Buzzelli et al., 1999). *Z. marina* respiration, related to daily production via temperature, was held at 0 and only increased when water temperatures were greater than 14 °C (Nejrup et al., 2008):

$$R_{zms} = C_{zms} * PR_{zm} * \left[0.00317 * (T_w + 0.105) + e^{(0.135 * T_w - 10.1)} \right] \quad (13)$$

where R_{zms} = respiration and PR_{zm} = *Z. marina* production (day^{-1} ; Buzzelli et al., 1999).

In addition to mortality and respiration *Z. marina* production was also lost through translocation (T_d) to the roots at a constant rate (day^{-1} ; Table 5-2). Epiphyte grazing was also held constant:

$$G_{epi} = C_{epi}^2 * K_{gepi} \quad (14)$$

where G_{epi} = epiphyte grazing and K_{gepi} = epiphyte grazing constant (day^{-1} ; Buzzelli et al., 1999).

Z. marina root and rhizome respiration was based on an Arrhenius relationship between respiration and water temperature (Bach, 1993). Respiration at an optimum temperature of 22 °C was scaled to daily temperatures with an Arrhenius constant (Θ_{zr}):

$$R_{zmr} = C_{zmr} * RR_{zmr} * \Theta^{(T_w - 22)} \quad (15)$$

(Buzzelli et al., 1999). Root mortality was computed as a constant fraction of biomass which increased after water temperatures became stressful (temperatures > 25 °C) to *Z. marina* in June of each model run (Setchell, 1929; Table 5-2).

Reproduction Model

Seeds were produced via flowering shoots and a carbon to shoot density conversion ($Veg_{C:D}$) based on *Z. marina* above ground biomass samples collected from GI in 2006 and 2007 ($n = 560$; Jarvis Chapter 4; Tables 5-1 and 5-3). Flowering was limited by water temperature and day and was based on the optimum conditions for *Z. marina* flowering observed in the York River (Silberhorn et al., 1983). When water temperatures were < 21 °C and Julian Day was < 182 (July 1) then 10 % of total shoot density was converted to flowering shoots (Silberhorn et al., 1983). In addition, flowering was initiated only when vegetative shoots dominated the *Z. marina* above ground carbon pool as *Z. marina* seedlings in the Chesapeake Bay do not flower during their first year of growth (Silberhorn et al., 1983). Subsequent loss of flowering shoots was considered to be inherently included in the above ground biomass mortality term.

Seed-bank densities were derived from the product of total flowering shoot densities and the average number of seeds per reproductive shoot ($Seeds_{tot}$, Table 5-3). Once produced, seeds were then deposited into the sediment seed-bank. While in the seed-bank a portion of the seeds were removed via mortality and predation (Table 5-3; Fishman and Orth, 1996). The number of germinable seeds remaining in the seed-bank was further reduced by a loss of viability (V_{seeds} ; Table 5-3). The seeds remained in the seed-bank until water temperatures decreased below 20 °C as this is when germination is initiated in Chesapeake Bay *Z. marina* populations (Moore et al., 1993). Due to the transient nature of Chesapeake Bay seed-banks those seeds that did not germinate by day 365 (December 31) were then lost from the system via seed mortality (Orth et al., 2000; Jarvis Chapter 3).

Germination of viable seeds ($Seeds_{via}$) was determined by a relationship between sediment organic content (SO, %) and seed burial depth (BD, cm) which was held constant at 3 cm:

$$Seeds_{germ} = \left(\frac{1}{1 + e^{(-0.1432 + (1.1261 * BD) + (-1.3964 * SO))}} \right) * Seeds_{via} \quad (16)$$

(Jarvis Chapter 2). Once germinated seedlings were then converted back to above and below ground carbon values (Table 5-3). When above ground *Z. marina* biomass was < 0.44 g C m⁻² then all above and below ground seedling biomass (T_{czmss} , T_{czmsr} , Table 5-1; Figure 5-2) was transferred over to the vegetative shoot and root stocks. If vegetative shoot carbon was > 0.44 g C m⁻² then seedling mortality was 100 %. This relationship was based on the inhibitory effect of shading by established vegetative above ground biomass on the survival of seedlings (Phillips et al., 1983; Robertson and Mann, 1984).

Seedling biomass was not tracked separately through the first year of growth due to a lack of information on seedling parameters.

Calibration, Validation, and Testing

Parameter values for epiphytes and *Z. marina* variables were selected from the literature and revised to increase model fit to calibration data within ecological limits (Table 2; Madden and Kemp, 1996; Buzzelli et al., 1999; Cerco and Moore, 2001). Due to the lack of information on *Z. marina* seedling production, parameter estimates for all variables except density to carbon ratios were assumed to be identical to vegetative shoots. Based on *in situ* data, initial above ground biomass values on April 1 were 6.68 g C m⁻² and 0.77 g C m⁻² at GI and GP respectively. Below ground biomass started at 9.21 g C m⁻² for GI and 0.94 g C m⁻² for GP. Initial epiphyte concentration was 0.25 g C m⁻² for both sites. Seed-bank densities and seedling biomass (above and below ground) started at 0. The model was calibrated to water column, sediment, and *Z. marina* data collected from GI bi-weekly to monthly from April to October in 2006 and 2007 (Moore unpublished data, Jarvis Chapter 4). Due to a lack of data on epiphytic biomass during this time period, epiphyte values were compared solely to literature values (Cerco and Moore, 2001). Comparisons were made between computed and observed values on a monthly basis.

Once calibrated, the model was then validated using water quality, sediment, and macrophyte data collected in 2006 and 2007 at GP (Jarvis Chapter 4). All model parameters were identical to the base model; however, forcing functions were based on GP data (<http://www2.vims.edu/vecos/>). Model validation was conducted both

graphically and statistically when possible. The sensitivity of base model conditions to all parameter estimates and forcing functions was analyzed by sequentially varying values by ± 5 , 10, and 20 %. The percent change in all state variables between the base model and sensitivity simulations was then calculated and tests that resulted in > 10 % change in state variable concentrations were considered to have the greatest impact on model results (Tables 5-4 and 5-5).

Model Scenarios

After calibration, the base model was extended to run from April 1, 2005 to December 31, 2007 for both the downriver (GI) and upriver (GP) sites. The model was extended to include 2005 in order to simulate the recovery of both sites after the fall 2005 decline. Forcing function data for 2005 were also collected from the CBNERRVA water quality monitoring network (<http://www2.vims.edu/vecos/>).

To quantify the potential role of seeds and seedlings in the recovery and re-establishment of *Z. marina* beds, the model was run with and without sexual reproduction. In addition, the role of the sediment seed-bank in providing resiliency to established beds to repeated stress events was quantified by increasing water temperatures in 1 °C increments from ambient (base model) conditions to ambient + 5 °C. Finally, the singular and combined effects of light and temperature stress on *Z. marina* reestablishment due to site differences in water column light attenuation were quantified by comparing GI *Z. marina* bed resiliency to temperature stress (ambient to ambient + 5 °C) under both GI and GP light attenuation conditions. Changes in above and below

ground *Z. marina* production, seed-bank density, viable seed output, and seedling density were quantified for all runs and compared to observed values.

Results

Model Calibration

Based on field observations *Z. marina* above ground biomass (reported as mean \pm S.E.) in 2006 and 2007 at GI ranged seasonally from $2.42 \pm 0.77 \text{ g C m}^{-2}$ to $38.58 \pm 8.32 \text{ g C m}^{-2}$ while the model output ranged from 0.28 g C m^{-2} to 38.49 g C m^{-2} (Figure 5-5). Overall the model captured seasonal trends in above ground biomass. The model was the most accurate in describing the initial recolonization of GI between April and July 2006 with an average percent error between the model and field data of $30 \pm 8 \%$. Following the maximum peak in above ground biomass the model diverged substantially from field measurements and the percent error from August to October averaged $87 \pm 3 \%$. The divergence during this time period was due to an under prediction of above ground biomass in the model. A similar pattern in model prediction was observed in 2007 with percent error from April to July 2007 decreasing slightly to $22 \pm 12 \%$ and to $63 \pm 6 \%$ in August to December 2007 (Figure 5-5).

Below ground biomass also varied seasonally, but to a lesser extent than above ground biomass. GI observed below ground biomass ranged from $9.21 \pm 2.08 \text{ g C m}^{-2}$ to $75.67 \pm 10.25 \text{ g C m}^{-2}$ while the model output ranged from 4.10 g C m^{-2} to 38.47 g C m^{-2} (Figure 5-5). Model predictions of belowground biomass were similar to observed values in 2006 with the average percent error of $33 \pm 4 \%$. The model was less accurate in predicting 2007 below ground biomass as the average percent error increased to

41 ± 11 %. As with above ground biomass, the model under predicted below ground biomass throughout the calibration period.

In 2007 observed seed production averaged $6,922 \pm 778$ seeds m^{-2} . The model predicted germinable seed densities of 13,034 seeds m^{-2} which is a percent error of 28 %. Maximum viable seed-bank densities predicted by the model were also greater than observed values. No viable seeds were found in the ambient sediment seed-bank in the GI site in 2006 or 2007. In the calibration model runs 0 seeds m^{-2} were produced in 2006 due to seedling dominance during the spring flowering period. However, in 2007 the model produced maximum seed viable seed-bank densities of 3,136 seeds m^{-2} .

Model Validation

The model accurately predicted lower overall above and below ground biomass values in GP *Z. marina* beds compared to GI (Figure 5-6). Observed GP above ground biomass values ranged from 0.44 ± 0.15 g C m^{-2} to 7.37 ± 1.93 g C m^{-2} in 2006 and from 1.08 ± 0.21 g C m^{-2} to 15.77 ± 7.10 g C m^{-2} in 2007. In comparison, model projected values ranged from 0.06 g C m^{-2} to 6.43 g C m^{-2} in 2006 and from 0.14 g C m^{-2} to 9.66 g C m^{-2} in 2007 (Figure 5-6). Similar to the base model runs, the validation runs were the most accurate in describing the initial recolonization in 2006 and the spring growth period (April to July) in 2007 and under-predicted biomass during the fall (August through October) period in both years. The model predictions for the spring growth period in 2007 were more accurate than 2006 spring growth with an average percent error of 27 ± 11 % in 2007 and 23 ± 11 % in 2006. Percent error from August to October

increased in both years with an average percent error of 56 ± 16 % in 2006 and 42 ± 21 % in 2007 (Figure 5-6).

Observed below ground biomass values were more variable at GP compared to GI with below ground biomass ranging from 1.50 ± 0.35 g C m⁻² to 11.44 ± 1.80 g C m⁻² in 2006 and from 3.92 ± 1.62 g C m⁻² to 26.21 ± 4.19 g C m⁻² in 2007. Model projected values were not as variable and ranged from 0.06 g C m⁻² to 6.43 g C m⁻² in 2006 to 0.41 g C m⁻² to 9.66 g C m⁻² in 2007 (Figure 5-6). Model accuracy in predicting belowground biomass was similar between years with an average percent error of 58 ± 12 % in 2006 and 59 ± 15 % in 2007. As with above ground biomass, the model under predicted below ground biomass throughout the validation period.

No flowering shoots were observed therefore no seeds were collected at GP in 2006 or in 2007. However, the model predicted germinable seed densities at 1,030 seeds m⁻². Maximum viable seed-bank densities predicted by the model were also greater than observed values. No viable seeds were found in the ambient sediment seed-bank in GP in 2006 or 2007. In the validation model runs 0 seeds m⁻² were produced in 2006; however, in 2007 the model produced maximum viable seed-bank densities of 302 seeds m⁻².

Sensitivity Analysis

Parameter Effects

Epiphyte biomass was most sensitive to changes in production and grazing (Table 4). Increasing grazing pressure by 5 % resulted in a significant (> 10% change) decrease of 17.9 % in epiphyte biomass while decreasing epiphyte production by 5 % resulted in a 33.4 % reduction in epiphyte biomass. Overall epiphytes were least responsive to

changes in the mortality rate and in respiration. Changes in epiphyte mortality rates up to $\pm 20\%$ did not result in a significant change in epiphyte biomass while altering respiration rates $< 20\%$ did not significantly affect epiphyte biomass. In general decreasing parameters had a greater effect on epiphyte biomass than increasing the parameters by the same margin (Table 5-4).

Z. marina shoot biomass was also most sensitive to changes in production (Table 5-4). By increasing *Z. marina* P_{max} by 5 % *Z. marina* biomass increased 53.5 %; similarly increases in *Z. marina* photosynthetic rate by 5 % resulted in a 47.6 % increase in *Z. marina* biomass. Changes in shoot to root translocation of carbon also had a significant effect on *Z. marina* biomass. A reduction in carbon translocation between shoots and roots of 5 % resulted in an increase in *Z. marina* biomass by 19.7 % (Table 5-4). Mortality and respiration rates did not have a significant effect on *Z. marina* biomass. Overall changes in parameter estimates which increased *Z. marina* shoot production (increased P_{max} and decreased translocation) had the greatest effect on *Z. marina* biomass.

The greatest change in *Z. marina* below ground biomass occurred when translocation of carbon from roots to shoots and shoot mortality decreased by 20 % (Table 5-4). *Z. marina* below ground biomass decreased by 23.9 % when translocation was reduced by 20 %. In addition a 20 % reduction in below ground mortality resulted in an 18.5 % increase in below ground biomass. Respiration did not significantly affect *Z. marina* below ground biomass. As with above ground biomass, below ground parameter estimates that increased below ground biomass (increased translocation and decreased mortality) had the greatest effect on *Z. marina* below ground biomass.

Z. marina seed-bank densities were affected to a greater extent by factors which reduced seed density (i.e. predation, mortality, and viability) than by factors that affected seed production (shoot carbon to density ratio; reproductive shoot densities; Table 5-4). Increasing and decreasing seed mortality, predation, and viability rates by 5 % resulted in similar changes of $\pm 18.5 - 24.3$ % in seed-bank density. Changes in flowering shoot density directly affected seed-bank density with a 10 or 20 % increase or decrease in flowering shoot density resulting in a similar increase or decrease in seed-bank density. Once in the seed-bank, seed germination was highly sensitive to the number of viable seeds (5 % increase in viable seeds = 43.9 % increase in germination) and seedling mortality (5 % decrease in mortality = 41.2 % increase in germination). Overall seed germination was more sensitive to increasing than decreasing seed viability while the effects of seed mortality were similar across analyses.

Forcing Functions

All state variables were sensitive to changes in temperature (Table 5-5). Epiphyte biomass increased 80 % with a 5 % increase in water temperature while a 5 % decrease resulted in a 46.1 % decrease in epiphyte biomass. All *Z. marina* state variables were more sensitive to decreases compared to increases in water temperature. Above and below ground shoot production increased 87.5 % and 58.3 % respectively with a 5 % decrease in water temperature. Seed-bank density was the most sensitive to increased water temperature as seed-bank density increased 461.7 % in response to a 5 % decrease in water temperature. Despite the large impact of decreased water temperature on seed-

bank density seed germination increased only 9.6 % when water temperatures decreased 5 % (Table 5-5).

Only epiphyte biomass and *Z. marina* above ground biomass were sensitive to changes in total available PAR₁ (Table 5-5). Epiphyte biomass decreased 15.5 % when light was reduced by 5 % while increasing PAR₁ by 5 % did not have a significant effect. Above ground *Z. marina* biomass increased 10.4 % when PAR₁ was increased by 10 %. All state variables had a similar response to changes in PAR₂ with only epiphyte and above ground *Z. marina* biomass showing significant sensitivity to this forcing function. As with PAR₁ epiphyte biomass decreased 15.9 % when PAR₂ was decreased 5 % and *Z. marina* above ground biomass increased 10.4 % when PAR₂ was increased 5 %. The similar response in epiphytes and above ground *Z. marina* biomass to changes in PAR₁ and PAR₂ indicates a lack of sensitivity to light in this model.

When PAR₃ was decreased by 5 % *Z. marina* above ground shoot biomass decreased 11.6 % resulting in a 13.2 % decrease in epiphyte biomass (Table 5-5). *Z. marina* below ground biomass was sensitive to PAR₃ only after it had been increased/decreased by 20 %. Below ground biomass responded similarly to a 20 % change in PAR₃ with an increase resulting in below ground biomass increasing by 18.9 % and a decrease resulting in a 18.3 % decrease in biomass. Sensitivity to PAR₃ was also found for the *Z. marina* seed-bank due to changes in reproductive shoot density. Seed-bank densities increased 12.3 % with a 5 % increase in PAR₃. Seed germination was also sensitive to increases in PAR₃ with a 5 % increase in PAR₃ resulting in a 56.0 % increase in seed germination.

Model Scenarios

Water Temperature

Model scenarios simulating the 2005 decline and first two years of recovery in York River *Z. marina* populations, quantified the effects of sexual reproduction, temperature stress, and the combined effect of light and temperature stress on *Z. marina* growth and survival. The negative effects of increased water temperature on above and below ground *Z. marina* biomass were evident during year one of the model run after an increase in water temperature of 1 °C significantly decreased (% change > 10 %) above and below ground biomass (Figure 5-7). During the first model run year biomass decreased between 34.2 – 58.0 % depending on site and mode of reproduction (vegetative only vs. mixed). Above ground biomass in subsequent model year runs also varied with mode of reproduction; however, the trend was similar with biomass decreasing with increasing water temperature. Maximum above ground biomass loss (100 %) occurred in year two of the model run at both sites when water temperatures were increased to ambient + 5 °C. Below ground biomass was more stable and decreased by 9.8 – 10 % with a 1 °C temperature increase which increased to 48.8 % when water temperatures were increased to ambient + 5 °C.

As with above and below ground biomass, total seed production, seed-bank density, and maximum viable seed density all decreased with increasing water temperature (Tables 5-6 and 5-7). Decreasing seed production was similar between sites with seed production decreasing 9.4 % in GI and 13.2 % at GP with a 1 °C increase in water temperature in 2005. Seed production decreased by 69.1 and 70.6 % at GI and GP respectively when water temperature was increased 5 °C. Although total seed production

under ambient conditions was the greatest of the three years with 13,034 seeds m⁻² and 1,656 seeds m⁻² produced in GI and GP respectively, total seed production decreased to 0 seeds m⁻² when water temperatures were increased to ambient + 1 °C (Table 5-6). Seed-bank and viable seed densities followed similar patterns to total seed production (Table 5-7)

Reproduction

Overall the model scenarios with the mixed mode of reproduction had greater above and below ground biomass than the vegetative only mode for year one and two regardless of temperature. Regardless of reproduction, all biomass was lost at both sites by year three once water temperature was increased 1 °C (Figure 5-8). The greatest difference between reproductive modes occurred after year one in the model run. For models running in vegetative mode a minimum increase in water temperature of 1 °C resulted in complete above ground biomass loss in year one. Without a seed input above ground biomass did not recover in year two or three regardless of site. While there was also a decline in all *Z. marina* parameters in the mixed reproduction model, when seed production was included beds at both sites recovered in year two. Despite this initial recovery period in year two, biomass never reached year one levels and all above ground biomass was lost by year three. Adding the mixed mode of reproduction (vegetative and sexual) to the model allowed for limited recovery; however, multiple years of stressful water temperatures resulted in complete loss of *Z. marina* regardless of reproductive mode.

Below ground biomass loss was limited compared to above ground biomass regardless of reproductive mode (Figure 5-8). For years one and two loss of below ground biomass was similar between mixed and vegetative reproductive modes with percent change between the two modes ranging from 0.0 to 32.8 %. By year three below ground biomass was completely absent from either site in the vegetative only models while the mixed models retained < 10 g C (Figure 5-8). By the end of year three below ground biomass was absent from all runs where temperature was increased by ≥ 1 °C.

Interactive Effects of Temperature and Light Stress

Overall model runs projected greater above ground biomass, below ground biomass, seed production, and seed germination at GI than at GP (Figures 5-7 and 5-8). While differences in water temperature between sites was $< 10\%$, GP had daily TSS values which were between 34.1 and 42.7 % greater and chlorophyll *a* concentrations between 30.0 and 52.1 % greater than GI depending upon the year (Figure 5-3). However, when the GI model was run with water column light attenuation values from GP, the model only projected a significant decrease in above ground biomass (32.74 % in 2006 and 91.13 % in 2007) under ambient water temperatures (Figure 5-9). The significant decrease under ambient conditions highlighted the interactive effects of temperature and light stress on *Z. marina* survival. There was no significant difference in above ground biomass between model comparisons of GI or GP water column light attenuation conditions in 2005 or when temperatures were increased above ambient conditions. Lowering the light levels at GI to GP conditions did not significantly

compound temperature stress and GI *Z. marina* populations continued to decline at rates similar to the model simulations with temperature stress only (Figure 5-9).

Discussion

Model Limitations

The model presented here reproduces the general observed trends in above and below ground *Z. marina* biomass in the York River following the 2005 decline; however, it does have several limitations. One of the greatest percent errors in the base model occurs due to a significant under-estimate (up to 84 ± 5 %) of fall *Z. marina* production. Under-estimation of fall production values may be attributed to the use of constant rates for translocation of carbon from *Z. marina* above ground to below ground biomass. As a temperate species, *Z. marina* enters a period of quiescence or limited production when water temperatures increase above 25 °C (Setchell, 1929). In the Chesapeake Bay water temperatures reach and remain above the 25 °C threshold from late July to early September significantly reducing *Z. marina* production and growth (Silberhorn et al., 1983). The lack of above ground production may inhibit carbon translocation to below ground biomass; however, as in Madden and Kemp (1996) and Cerco and Moore (2001), the exact relationship is unknown therefore translocation remained constant throughout all model runs possibly resulting in lower fall above ground biomass values. Defining the seasonality of the relationship between temperature and translocation and leaf sloughing within *Z. marina* plants is necessary to increase the accuracy of this model.

In addition, the total number of seeds produced was overestimated by the base model in 2007 by 85 %. While this is a large percent error, the number of seeds produced

within the bed during the 2007 model run (1,656 to 13,034 seeds m⁻²) was not outside of the reported range of perennial *Z. marina* seed production of 50 – 25,500 seeds m⁻² (Silberhorn et al., 1983; Harwell and Rhode, 2007; Lee et al., 2007). The over-estimation of *Z. marina* seed densities in the model prediction compared to *in situ* field measurements may be a reflection of the relatively sparse field sampling (monthly) compared to the finer model scenarios (daily). In addition, flowering shoot development is affected by a variety of factors including temperature, light, and predation (Setchell, 1929; Phillips et al., 1983; Silberhorn et al., 1983). Not all impacts are lethal to the flowering shoot; however, they may result in reduced seed production or lower seed viability (Harper, 1977). As the relationship between environmental factors and seed development is not defined, this remains a limitation of the model.

Once the model was expanded to include data from 2005 to 2007, the above ground biomass predictions for GP data were up to three times greater in the model run compared to observed values. The over-production of *Z. marina* biomass in the model resulted in an average percent error of 235.17 % in 2006 and 449.74 % in 2007 compared to observed values. The over-prediction is related to larger initial above ground biomass conditions from seed germination following the 2005 decline. Relationships between *Z. marina* seedling growth and survival and surrounding environmental conditions are not well defined. There is some evidence that seedlings respond similarly to temperature limitations when compared to established *Z. marina* plants (Bintz et al., 2003; Abe et al., 2008); however, there is little other information available on *Z. marina* seedlings or the effects of changes in habitat conditions on seedling growth and survival. Therefore, in this model, seedling functions were identical to vegetative shoot functions and may have

resulted in the over-production of above ground biomass in 2006 and 2007. Information on seedling physiology is required to increase the accuracy of the sexual reproduction component of this model.

Sexual Reproduction, Temperature, and Light

Model projections for *Z. marina* production following the 2005 decline and subsequent recovery period were more accurate when sexual reproduction was added compared to the traditional production model with vegetative reproduction only. Following the 2005 decline, in simulations where sexual reproduction is included, model results project an initial period of recovery in 2006 in all temperature treatments (Figures 5-7 and 5-8). Without the inclusion of sexual reproduction, there was no recovery following the 2005 decline regardless of temperature. However, the resiliency to temperature stress provided by sexual reproduction is limited as a 1 °C increase in temperature resulted in a reduction of biomass to 0.00 g C m⁻² by year three. Differences in water quality between sites also affected *Z. marina* above and below ground production, seed production, seed viability and seed germination. Under ambient temperatures, the combination of light and temperature stress present at the upriver site (GP) resulted in overall lower production and resiliency to declines compared to the down river site (GI; Figure 5-9). However; when water temperatures were increased 1 °C temperature stress resulted in a complete reduction of above ground biomass regardless of site (Figure 5-9). The results of all model scenarios highlight the need to incorporate sexual reproduction into *Z. marina* ecosystem models, the projected sensitivity of

established *Z. marina* beds to consecutive years of stress, and the negative effects of multiple stressors on *Z. marina* recovery.

Following the 2005 decline, re-establishment of both GI and GP was initiated by seed germination and seedling establishment in the mixed reproduction model. These results are similar to the observed recovery in the York River in 2006 where seedlings constituted > 80 % of total shoot density at both GI and GP (Jarvis, Chapter 4). Similar results were seen following a large scale decline in the Odense Fjord, Denmark where up to up to 96 % of all observed shoots were seedlings during the initial recovery period (Greve et al., 2005). While seed germination is necessary for the success of sexual reproduction, seedling establishment and survival is a limiting factor for many autotrophs including *Z. marina* (Harper et al., 1977; Harrison, 1993). In the Thau Lagoon, France during the first 6 months following a massive germination event seedling survival rates were 80 % (Plus et al., 2003). Similar results were seen in the model presented here where above ground biomass increased until summer water temperatures became limiting. The reduction in above ground biomass in response to temperature is also observed in established *Z. marina* beds in the lower Chesapeake Bay (Orth and Moore, 1986).

Similarly when seeds were not produced (as in the spring and summer of 2006 in all model runs) there was no recovery following the second consecutive decline during all temperature scenarios in both sites (Figure 5-8). In the mixed model flowering shoot production was absent during the spring of 2006 due to the prevalence of seedlings which do not flower during their first year of growth in the Chesapeake Bay (Silberhorn et al., 1983). Similarly, in the Thau Lagoon no reproductive shoots were produced during the

first year of re-establishment following the 2001 decline (Plus et al., 2003). A significant increase was observed in the same system during the second year of recovery when 75 % of total shoots were reproductive. The model described here did not increase flowering rates at any point in the three year run. However, an increase in flowering shoot production in the York River two years post-decline was not observed and reproductive shoot densities remained within the 10 – 20 % of total shoot density recorded for the York River (Silberhorn et al., 1983; Harwell and Orth, 2002).

Without the production of flowering shoots in the model in 2006 seeds were not produced, the seed-bank was not replenished, and the resiliency provided by the seed-bank was lost. On average, perennial *Z. marina* seed production ranges between 50 – 25,500 seeds m⁻² (Silberhorn et al., 1983; Harwell and Rhode, 2007; Lee et al., 2007). The model projected between 4,033 to 13,034 seeds m⁻² in 2005 and between 0 and 1,656 seeds m⁻² in 2007 depending upon site and water temperature (Table 5-6). However, seed-bank density is not a direct reflection of yearly seed production (Baskin and Baskin, 1998). *Z. marina* seeds are lost to dispersal (Källström et al. 2008), predation (Fishman and Orth, 1996), and mortality (Morita et al., 2007). In Ago Bay Japan up to 72 % of total seeds produced are lost from the system annually (Morita et al., 2007). In this model seeds were subject to mortality and predation after they were produced. As a result in this model seed-bank densities were 69.1 % less than total seed production. Literature values for *Z. marina* sediment seed-bank densities range from 0 – 25,746 seeds m⁻² (Harwell and Orth, 2002; Morita et al., 2007; Lee et al., 2007). Maximum model seed-bank densities were found in 2005 and were well within the literature values (max 14,970 seeds m⁻²). Projected seed-bank densities in 2006 were

similar to York River observations of 0 ± 0 seeds m^{-2} however projected seed-bank densities were significantly greater in 2007 compared to observed York River values (0 ± 0 seeds m^{-2} , Jarvis Chapter 4).

The discrepancy between model projected seed-bank values and observed values may be explained by the patchy distribution of seeds within established *Z. marina* beds (Harwell and Orth, 2002). The development of reproductive shoots within *Z. marina* beds is not homogeneous (Harwell and Rhode, 2007). Seeds may still be attached to reproductive shoots that have detached from the main vegetative shoot and may be distributed in clumps rather than as individual seeds (Terrados, 1993). As a result ambient seed-banks are extremely patchy and hard to quantify (Harwell and Orth, 2002). The model does not have a spatial component therefore the patchy distribution of seeds is not taken into account and all seeds are easily accounted for possibly resulting in the greater predicted seed-bank densities.

In all model runs where temperature was increased, there was no re-establishment following periods when seeds were not produced. The decline occurred in 2006 for the runs with only vegetative reproduction, for the mixed reproduction runs the decline occurred a year later in 2007. In the York River, *Z. marina* seed-banks are transient and need to be replenished on a yearly basis because seeds are unable to maintain viability for periods longer than 6 - 12 months (Orth et al. 2000; Harwell and Orth, 2002; Jarvis Chapter 3). Therefore, any remaining non-germinating seeds from 2005 would not be able to germinate in 2006 due to a loss of viability. Without the resiliency provided by the sediment seed-bank neither bed was able to recover from a second consecutive year of stressful conditions.

Within the model, *Z. marina* above and below ground production was able to recover from the simulated 2005 decline under ambient water quality conditions. These projections were supported by the observed recovery at both GI and GP in 2006 and 2007 when summer water temperatures were not limiting (Jarvis Chapter 4). However, once temperatures were increased 1 °C *Z. marina* biomass declined completely in year one in the vegetative only model and a year later in the mixed model (Figure 5-8). These results highlight the projected sensitivity of York River *Z. marina* populations to extended periods of increased water temperatures. *Z. marina* populations in the York River are located near the southern limit of the species western Atlantic distribution and are stressed by summer water temperatures that can reach as high as 30 °C during extreme warming periods (Short and Moore, 2006; Moore and Jarvis, 2008). As water temperatures increase above 20 °C *Z. marina* respiration increases at a greater rate than photosynthesis causing stress and eventually mortality when water temperatures are greater than 25 °C (Marsh et al., 1986; Nejrup et al., 2008). As a consequence, summer water temperatures are limiting and result in seasonal declines in *Z. marina* biomass (Orth and Moore, 1986). By increasing year round water temperatures by 1 °C all above ground biomass was removed from the model before cooler fall temperatures could release *Z. marina* populations from summer water temperature limitation. The response of temperature stressed *Z. marina* shoots to increased water temperature requires further research as global water temperatures are predicted to rise at an increasing rate over the next century (Stern, 2006).

Z. marina populations within the Chesapeake Bay are also stressed by low light conditions (Dennison et al., 1993; Moore et al., 1996). This was most evident in the

upriver GP site where light was reduced compared to the downriver GI site (Figure 5-3). Therefore this site was likely not as resilient to increases in water temperature as the down river site and the *Z. marina* beds were not able to recover in the first year post the 2005 decline with a mixed reproduction model once temperatures were increased above ambient + 3 °C. These results highlight possible mechanisms behind reported limitations in *Z. marina* restoration success and natural recovery of *Z. marina* beds in the York River which have been attributed to seasonal pulses in turbidity (Moore et al., 1996). When low light conditions are combined with increased water temperature the effects become lethal to *Z. marina* survival (Moore and Jarvis, 2008).

Conclusions

Although the model presented here is designed with *Z. marina* seedling above and below ground biomass as separate state variables, it currently moves all seedling biomass directly to the vegetative shoot biomass pool as soon as it is produced. To gain a better understanding of the relative amount of biomass produced by seedlings compared to surviving vegetative material, this component of the model needs to be further refined and run separately for at least one year. By quantifying the growth and survival of *Z. marina* seedlings under a variety of environmental conditions over a longer period of time, the role of seedlings in the recovery of perennial *Z. marina* beds can be elucidated. In addition, the refinement of seedling state variables may expand the application of this model to include annual/semi-annual as well as perennial *Z. marina* populations.

Overall the model results presented here highlight a need for the inclusion of sexual reproduction within *Z. marina* models. This directly applies to models attempting

to project loss and recovery processes within *Z. marina* communities, as sexual reproduction plays a large role in recovery from large scale declines. In addition, model projections indicate that current York River *Z. marina* populations are near the limit for temperature stress and increases in water temperatures as small as 1 °C may have large impacts on *Z. marina* survival. The loss of *Z. marina* due to temperature stress or temperature and light stress can be ameliorated by sexual reproduction; however this resiliency is limited by seed production and seed-bank viability and multiple concurrent years of stress may result in long term loss of *Z. marina*. Further research into all aspects of sexual reproduction, seedling establishment, growth, and survival in temperate *Z. marina* beds is required to advance the understanding of loss and recovery patterns in Chesapeake Bay *Z. marina* beds.

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Table 5-1. Governing equations for (1) epiphyte biomass (C_{epi} ; $g C m^{-2} day^{-1}$); (2) *Z. marina* vegetative shoot biomass (C_{zms} ; $g C m^{-2} day^{-1}$); (3) *Z. marina* vegetative root/rhizome biomass (C_{zmr} ; $g C m^{-2} day^{-1}$); (4) *Z. marina* seedling shoot biomass (C_{zmss} ; $g C m^{-2} day^{-1}$); (5) *Z. marina* seedling root/ biomass (C_{zmsr} ; $g C m^{-2} day^{-1}$); (6) *Z. marina* seed-bank density (Zm_{seeds} ; seeds m^{-2}); and (7) *Z. marina* seedling density (Zm_{sd} ; seedlings m^{-2}). Terms include P = production; M = mortality; G = grazing; R = respiration; T_d = translocation down; T_{czmss} = transfer of seedling biomass to vegetative shoot biomass; T_{czmsr} = transfer of seedling root/rhizome biomass to vegetative root/rhizome biomass; $Seeds_{germ}$ = germinated seeds; $Seeds_{tot}$ = total seeds produced; $Seeds_{via}$ = viable seeds; P_{seeds} = seed predation; Zm_{sd} = germinated seedling density.

Differential Equations

$$C_{epi} = C_{epi} (t - dt) + (P_{epi} - M_{epi} - G_{epi} - R_{epi}) * dt$$

$$C_{zms} = C_{zms} (t - dt) + (P_{zms} + T_{czmss} - M_{zms} - R_{zms} - T_d) * dt$$

$$C_{zmr} = C_{zmr} (t - dt) + (T_d + T_{czmsr} - M_{zmr} - R_{zmr}) * dt$$

$$Zm_{seeds} = Zm_{seeds} (t - dt) + [((Seeds_{tot} - M_{seeds} - P_{seeds}) - Seeds_{via}) - Seeds_{germ}] * dt$$

$$Zm_{sd} = Zm_{sd} (t - dt) + (Seeds_{germ} - M_{zmsd}) * dt$$

$$C_{zmss} = C_{zmss} (t - dt) + (P_{zmss} + (T_{czmss} * Seeds_{C:DAG}) - T_{czmss} - T_d - M_{zmss} - R_{zmss}) * dt$$

$$C_{zmsr} = C_{zmsr} (t - dt) + (T_d + (T_{czmsr} * Seeds_{C:DBG}) - T_{czmsr} - M_{zmsr} - R_{zmsr}) * dt$$

Table 5-2. Parameter estimates for the *Z. marina* production model. References: 1 = calibration, 2 = Buzzelli et al., 1999, 3 = Cerco and Moore, 2001, 4 = Madden and Kemp, 1996; 5 = Bach, 1993; 6 = Harwell and Orth, 1996.

Abbrev.	Description	Units	Value	Ref
JD	Julian Day	d ⁻¹	0-365	
MR _{epi}	epiphyte mortality constant	d ⁻¹	0.0085	1
K _{gepi}	epiphyte grazing constant	g C ⁻¹ day ⁻¹	0.01	1
BMR _{epi}	epiphyte basal metabolic rate	day ⁻¹	0.047	2
KtB _{epi}	epiphyte respiration constant	°C ¹	0.069	2
Topt _{epi}	epiphyte optimum temperature for metabolism	°C	25	2
Khn _{epi}	epiphyte N half saturation constant	μmol N m ⁻³	1.79E-09	3
Khp _{epi}	epiphyte P half saturation constant	μmol P m ⁻³	7.14E-11	3
Khnw _{zm}	<i>Z. marina</i> N half saturation constant water	μmol N m ⁻³	7.14E-10	3
Khns _{zm}	<i>Z. marina</i> N half saturation constant sediment	μmol N m ⁻³	2.86E-09	3
Khpw _{zm}	<i>Z. marina</i> P half saturation constant water	μmol P m ⁻³	4.35E-10	3
Khps _{zm}	<i>Z. marina</i> P half saturation constant sediment	μmol P m ⁻³	7.14E-09	3, 4
KPAR _{epi}	epiphyte PAR half saturation constant	μE m ⁻² s ⁻¹	90	4
KPAR _{zm}	<i>Z. marina</i> PAR half saturation constant	μE m ⁻² s ⁻¹	57.5	3
MR _{zms}	<i>Z. marina</i> shoot mortality constant	d ⁻¹	0.007	1
Topt _{zm}	<i>Z. marina</i> optimum temperature for metabolism	°C	22.5	3
T _d	<i>Z. marina</i> shoot to root transfer	g C day ⁻¹	0.3	3
MR _{zmr}	<i>Z. marina</i> root mortality constant Jan - July	d ⁻¹	0.000085	1
	<i>Z. marina</i> root mortality constant July - Dec	d ⁻¹	0.0095	1
RR _{zmr}	<i>Z. marina</i> root respiration at 20 °C	d ⁻¹	0.0005	1
Θ _{zmr}	<i>Z. marina</i> root respiration constant	unit-less	1.25	5
PR _{seeds}	Seed Predation	shoots d ⁻¹	0.5	5
MR _{zmseeds}	Seed mortality constant	seeds d ⁻¹	0.1	1
VR _{seeds}	Seed viability constant	seeds d ⁻¹	0.4	1

Table 5-3. Parameter estimates for the *Z. marina* reproduction model. References: 1 = calibration, 2 = Silberhorn et al., 1983; 3. = Harwell, 2000; 4 = Fishman and Orth, 1996

Abbrev	Description	Units	Value	Ref
Veg _{C:D}	<i>Z. marina</i> shoot carbon to density	g C shoot ⁻¹	0.0168	n = 560 shoots
ZmRS _{den}	reproductive shoot density	%	0-19	2
Seeds _{tot}	seeds per reproductive shoot	seeds shoot ⁻¹	7 - 11	3
P _{seeds}	seeds predation rate	day ⁻¹	0.33	4
M _{seeds}	seeds mortality rate	day ⁻¹	0.1	1
V _{seeds}	seeds viability rate	day ⁻¹	0.4	n = 100 seeds
Seedlings _{SD:C}	<i>Z. marina</i> seedling density to shoots	g C shoot ⁻¹	0.0374	n = 120 shoots
Seedling _{RD:C}	<i>Z. marina</i> seedling density to shoots	g C shoot ⁻¹	0.0384	n = 120 shoots

Table 5-4. Minimum sensitivity simulation ($\pm 5, 10, 20 \%$) for model parameters which resulted in significant variation ($\geq 10 \%$) of state variables relative to base model concentrations. Non-significant values are denoted with (--).

State Variable	Parameter	Min % Change
Epiphytes	PR_{epi}	-5
	P_{max}	± 5
	K_{gepi}	+ 5
	MR_{epi}	--
	BMR_{epi}	± 5
	KtB_{epi}	± 20
<i>Z. marina</i> shoots	PR_{zm}	± 5
	P_{max}	± 5
	T_{czms}	--
	MR_{zms}	--
	R_{zms}	± 20
	T_d	± 5
<i>Z. marina</i> Root/Rhizome	T_d	-10
	T_{czmsr}	--
	MR_{zmr}	± 20
	RR_{zmr}	--
	R_{zmr}	--
	$Veg_{D:C}$	-10
Seed-bank	F_{sden}	± 10
	MR_{seeds}	± 5
	PR_{seeds}	+ 5
	VR_{seeds}	± 5
Seed Germination	V_{seeds}	± 5
	M_{sd}	± 5

Table 5-5. Minimum sensitivity simulation ($\pm 5, 10, 20 \%$) for model parameters which resulted in significant variation ($\geq 10 \%$) of forcing functions relative to base model concentrations. Non significant values are denoted with (--).

Forcing Function	Parameter	% Change
Temperature	Epi	± 5
	Zm Shoots	± 5
	Zm Roots	± 5
	Seed-bank	± 5
	Seed Germination	-5
PAR ₁	Epi	-5
	Zm Shoots	+ 5
	Zm Roots	--
	Seed-bank	-20
	Seed Germination	--
PAR ₂	Epi	-5
	Zm Shoots	+ 5
	Zm Roots	--
	Seed-bank	--
	Seed Germination	--
PAR ₃	Epi	-5
	Zm Shoots	-5
	Zm Roots	± 20
	Seed-bank	+ 5
	Seed Germination	+ 5
Chlorophyll <i>a</i>	Epi	+ 5
	Zm Shoots	--
	Zm Roots	--
	Seed-bank	--
	Seed Germination	--
TSS	Epi	+ 5
	Zm Shoots	--
	Zm Roots	--
	Seed-bank	--
	Seed Germination	--

Table 5-6. Model projected maximum seed-bank densities between 2005 – 2007 for all temperature runs. Temperatures are in °C.

Date	Germinable Seed Density m⁻²					
	Ambient	Amb + 1	Amb + 2	Amb + 3	Amb + 4	Amb + 5
GI						
Fall 2005	13,034	11,805	8,842	6,232	4,986	4,033
Fall 2006	0	0	0	0	0	0
Fall 2007	9,677	0	0	0	0	0
GP						
Fall 2005	1,656	1,438	1,141	693	526	487
Fall 2006	0	0	0	0	0	0
Fall 2007	10,030	0	0	0	0	0

Table 5-7. Model projected maximum viable seed-bank densities between 2005 – 2007 for all temperature runs. Temperatures are in °C.

Year	Maximum Viable Seed Density m⁻²					
	Ambient	Amb + 1	Amb + 2	Amb + 3	Amb + 4	Amb + 5
GI						
Fall 2005	4,224	3,826	2,866	2,020	1,629	1,307
Fall 2006	0	0	0	0	0	0
Fall 2007	3,136	0	0	0	0	0
GP						
Fall 2005	537	466	370	224	170	158
Fall 2006	0	0	0	0	0	0
Fall 2007	302	0	0	0	0	0

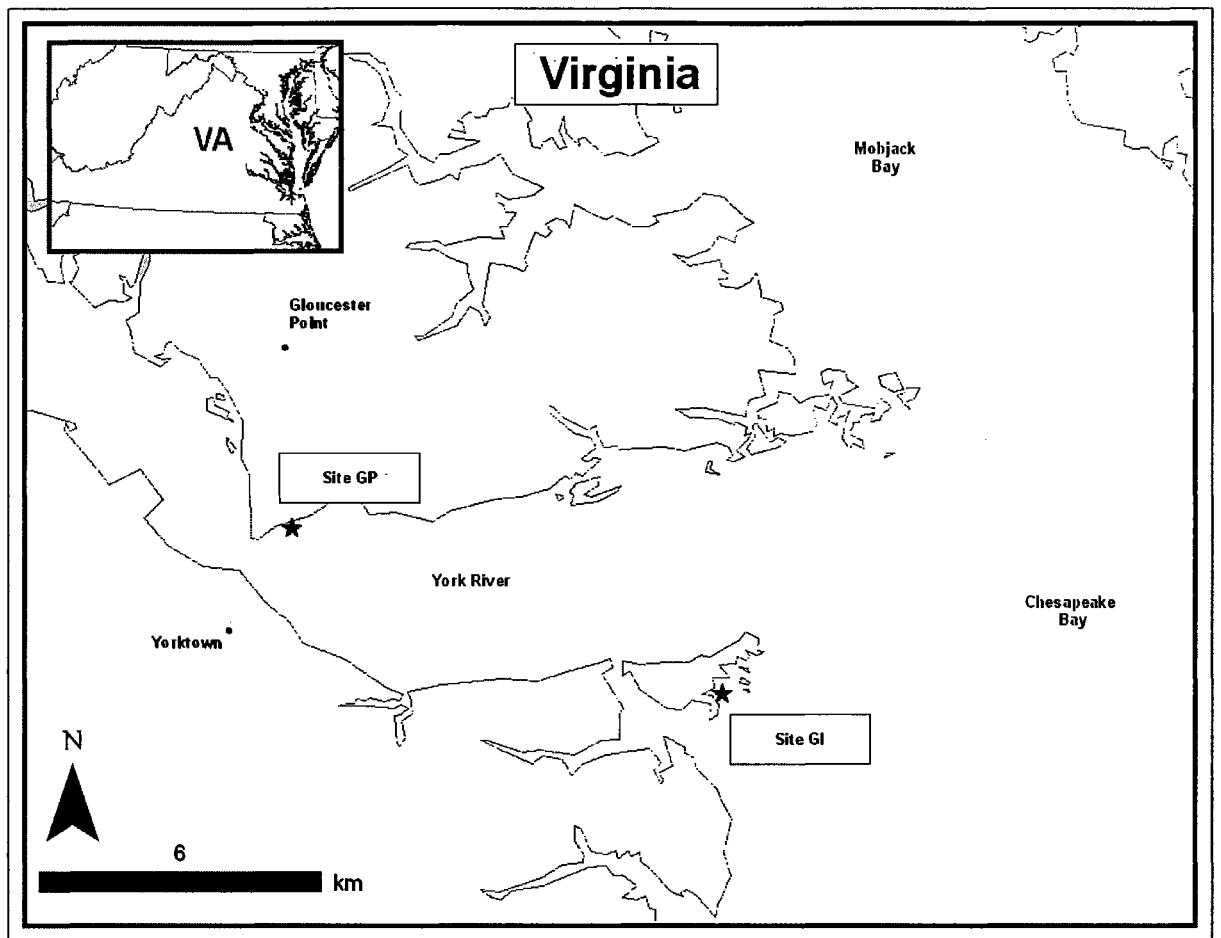


Figure 5-1. Lower York River Virginia site locations for all calibration and forcing function data collection. Goodwin Island (GI) is located at the mouth of the York River and Gloucester Point (GP) is located 10 km upriver. Site locations are denoted with a star.

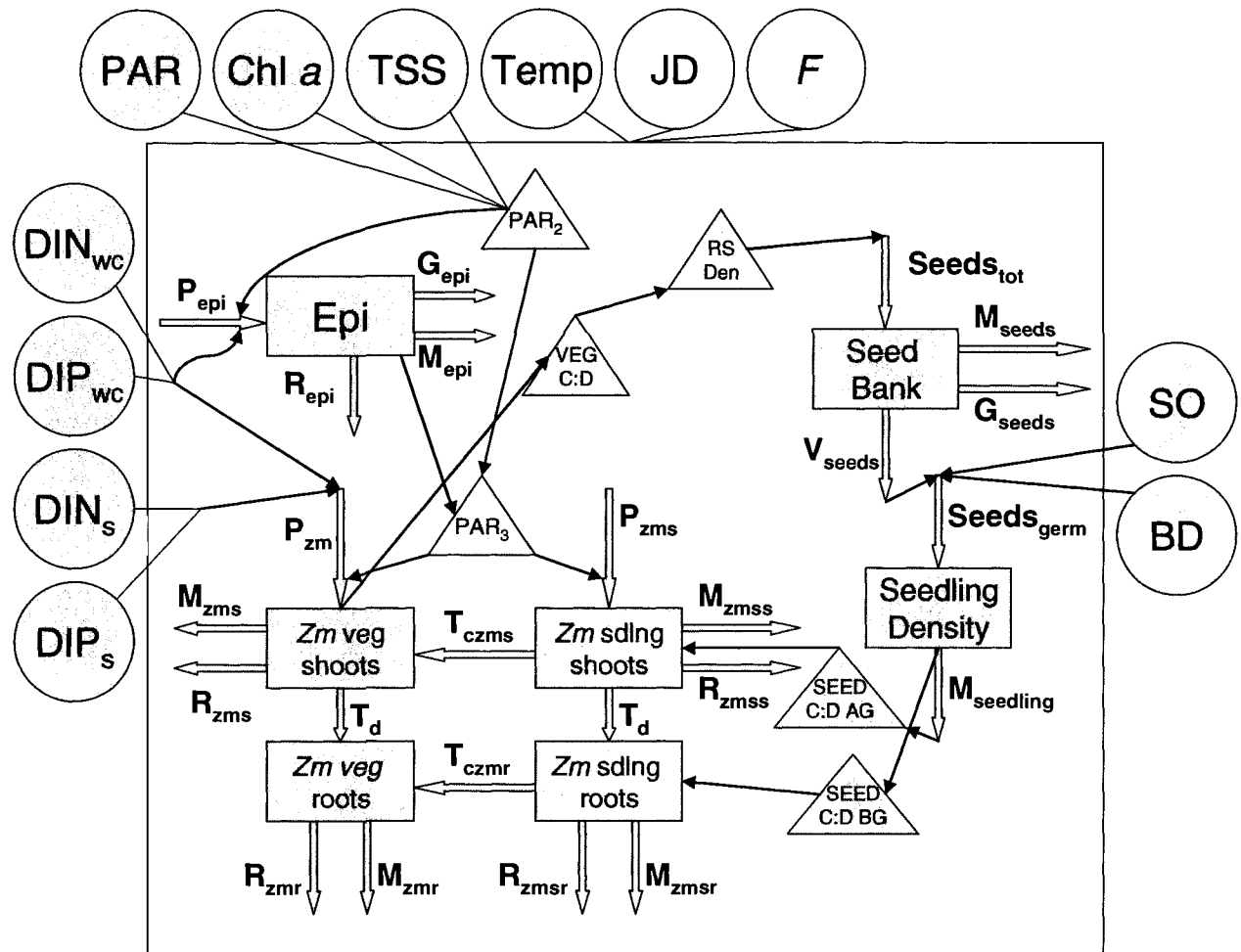


Figure 5-2. Conceptual diagram for *Zostera marina* production and sexual reproduction model. Circles = forcing functions, triangles = modifiers, squares = state variables, thick arrows = flows, and thin arrows = iterations. All variables defined in text. Temp, JD, and F affect multiple processes so are not connected to minimize diagram complexity.

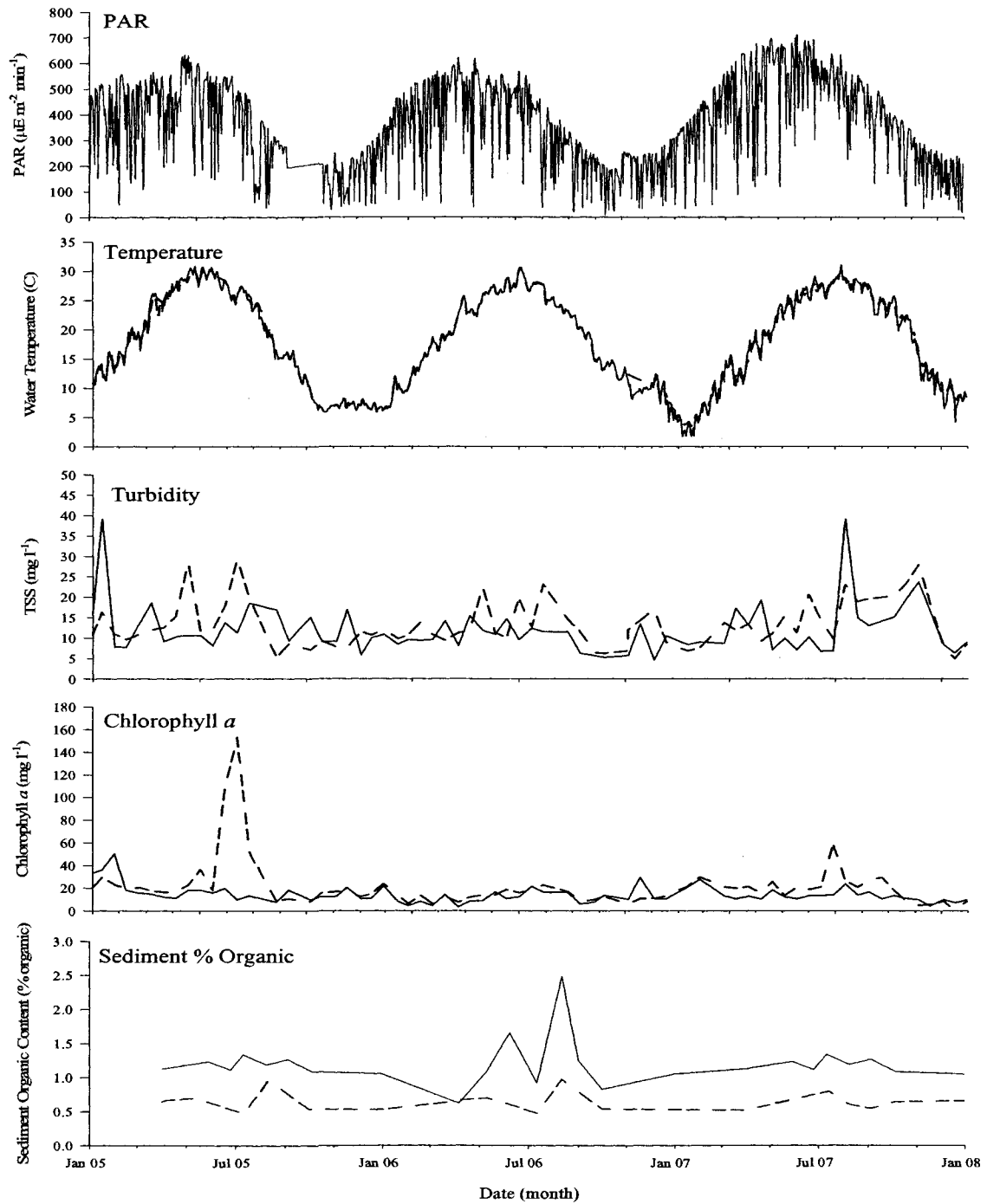


Figure 5-3. Forcing functions for Goodwin Island (solid line) and Gloucester Point (dashed line) for 2005 - 2007.

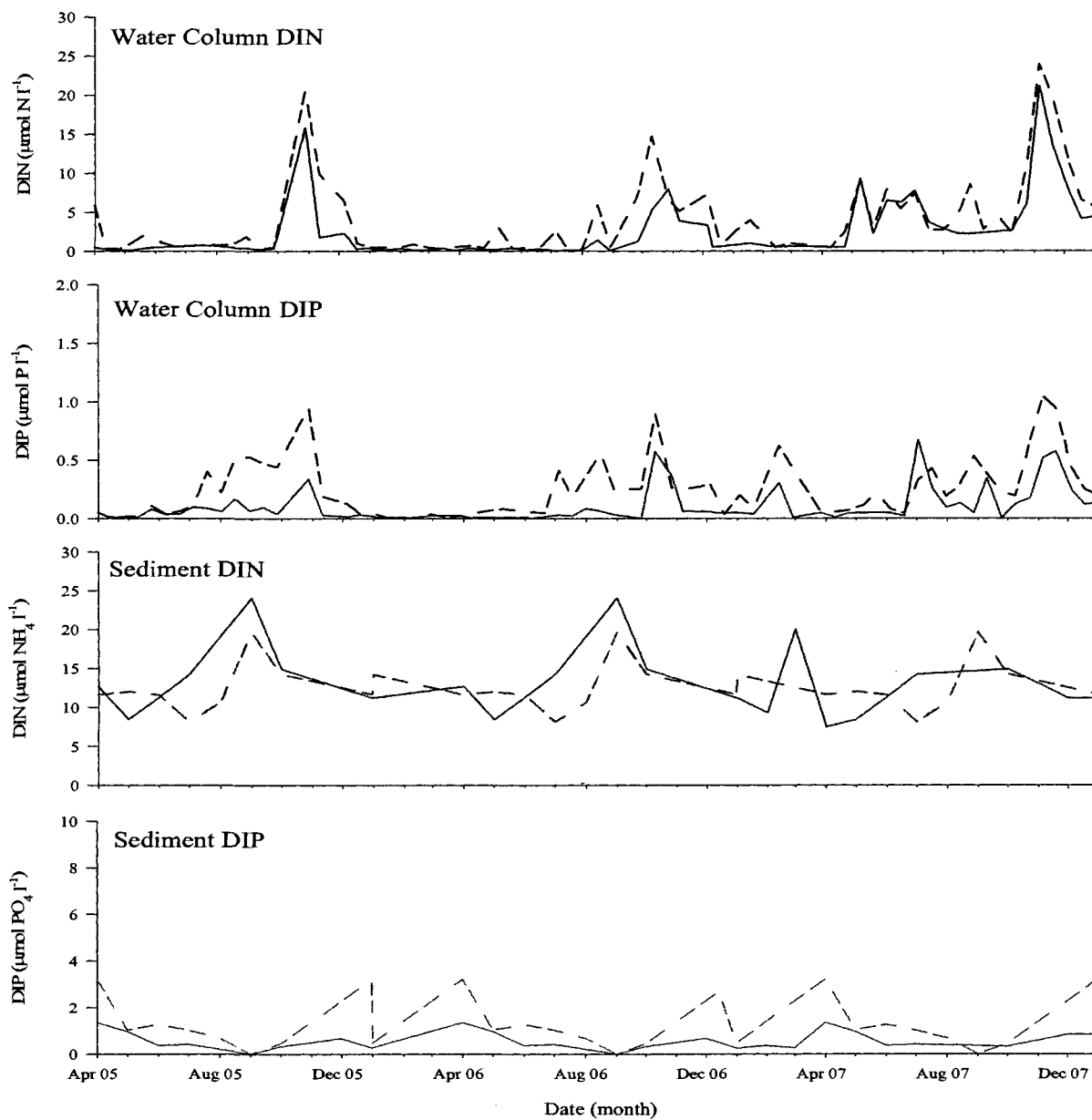


Figure 5-4. Water column and sediment nutrient forcing functions for Goodwin Island (solid line) and Gloucester Point (dashed line) for 2005 – 2007.

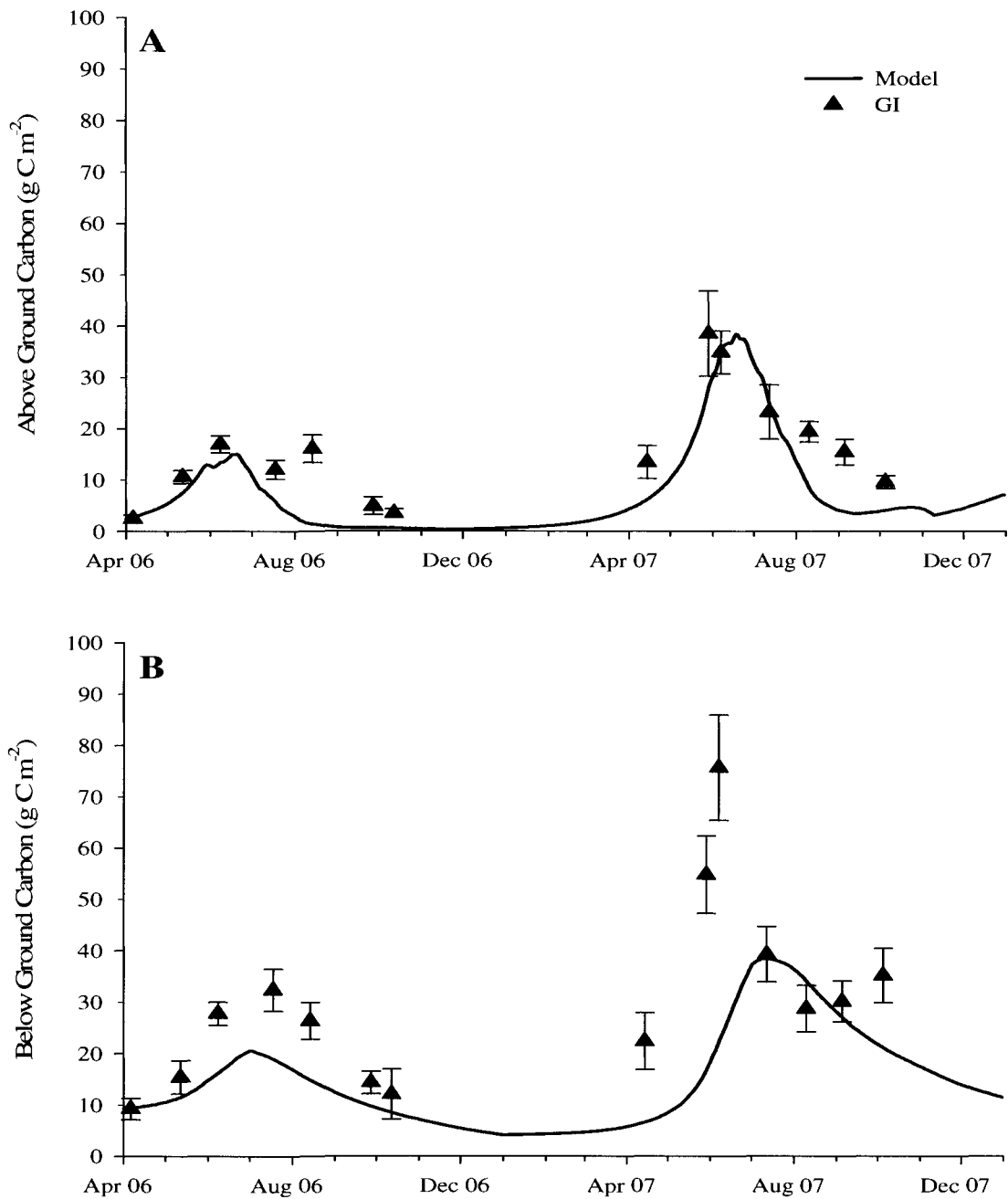


Figure 5-5. Calibration of *Zostera marina* above and below ground biomass model (black line) with observed GI data (red triangles). Observed data are given in monthly means \pm SE.

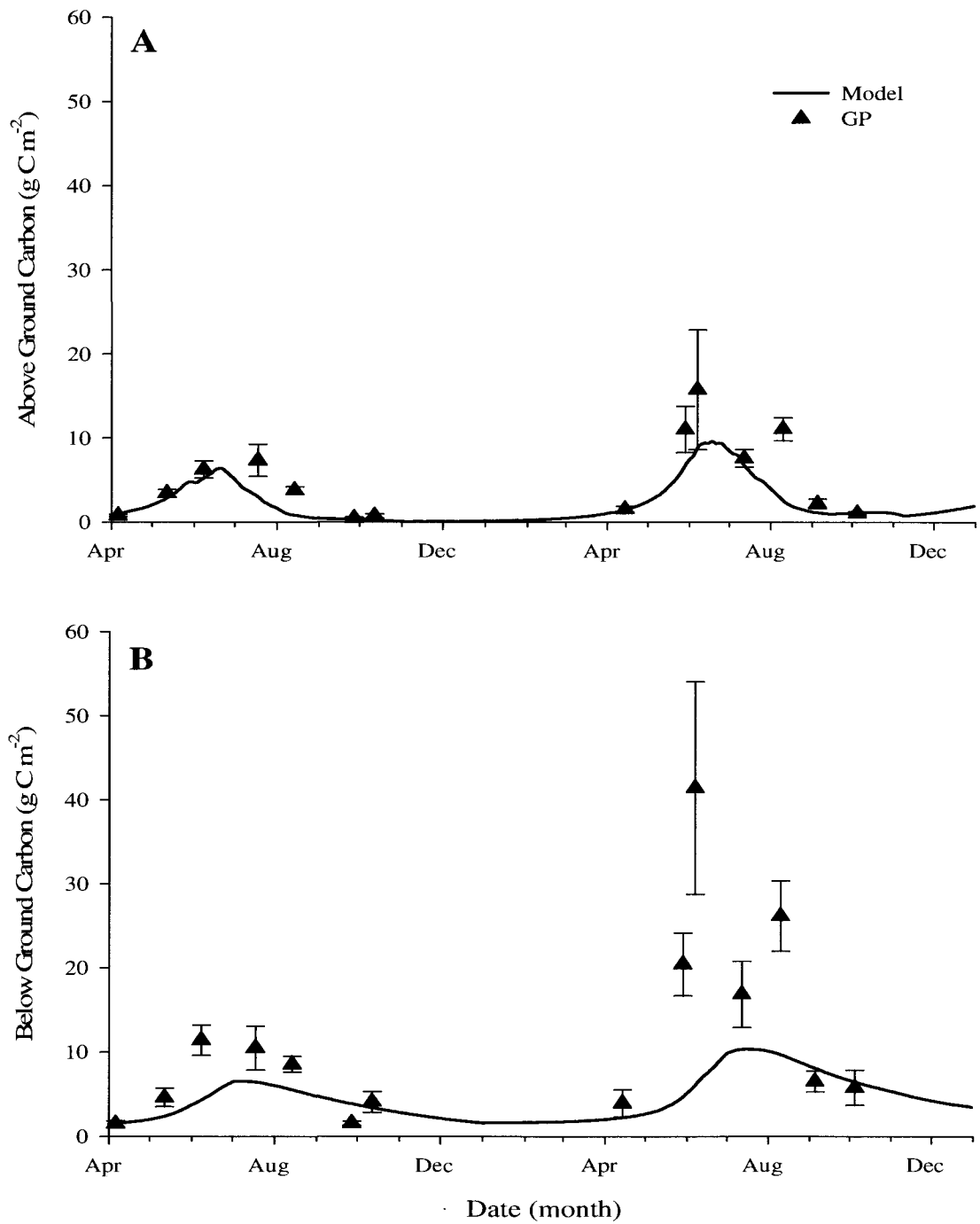


Figure 5-6. Validation of *Z. marina* above and below ground biomass model (black line) with observed GP data (red triangles). Observed data are given in monthly means \pm SE.

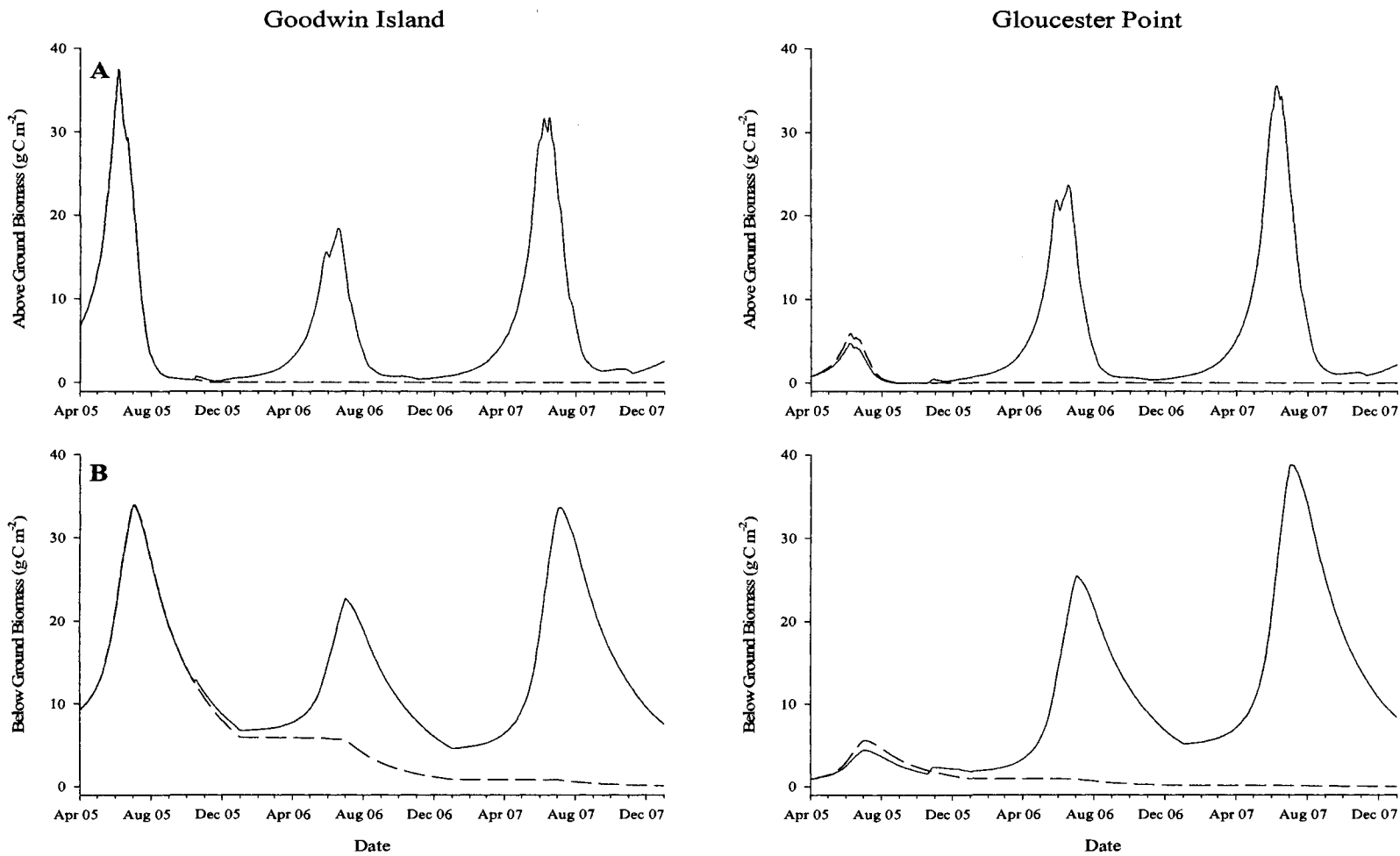


Figure 5-7. Goodwin Island and Gloucester Point (A) above ground biomass and (B) below ground biomass model projections with sexual reproduction (solid line) and without sexual reproduction (dashed line) for 2005 – 2007 under ambient temperatures.

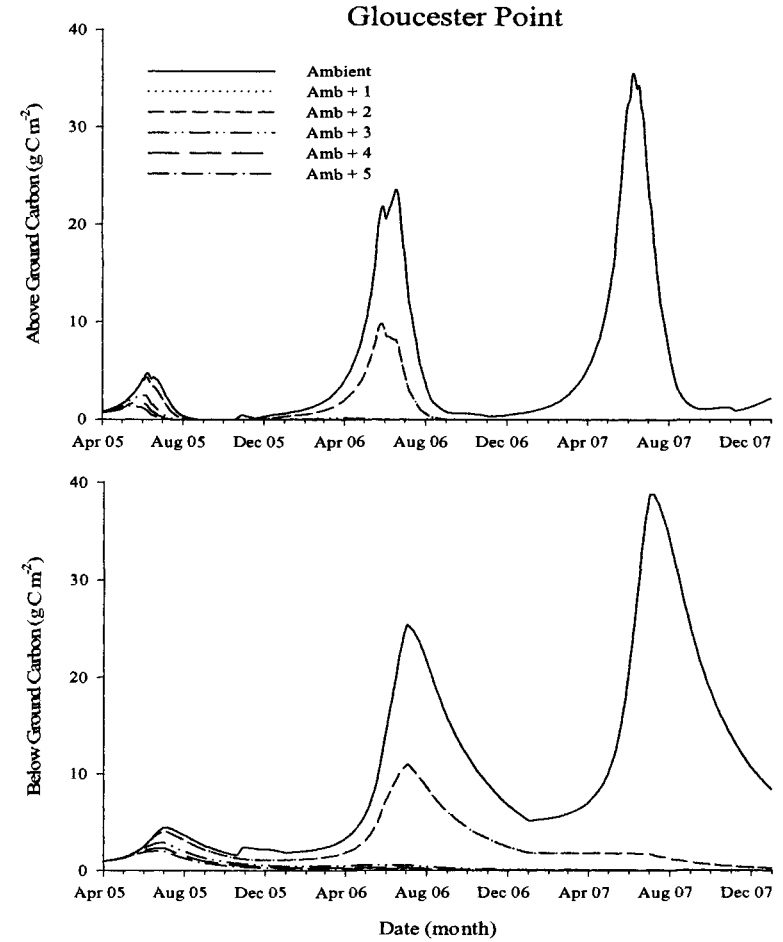
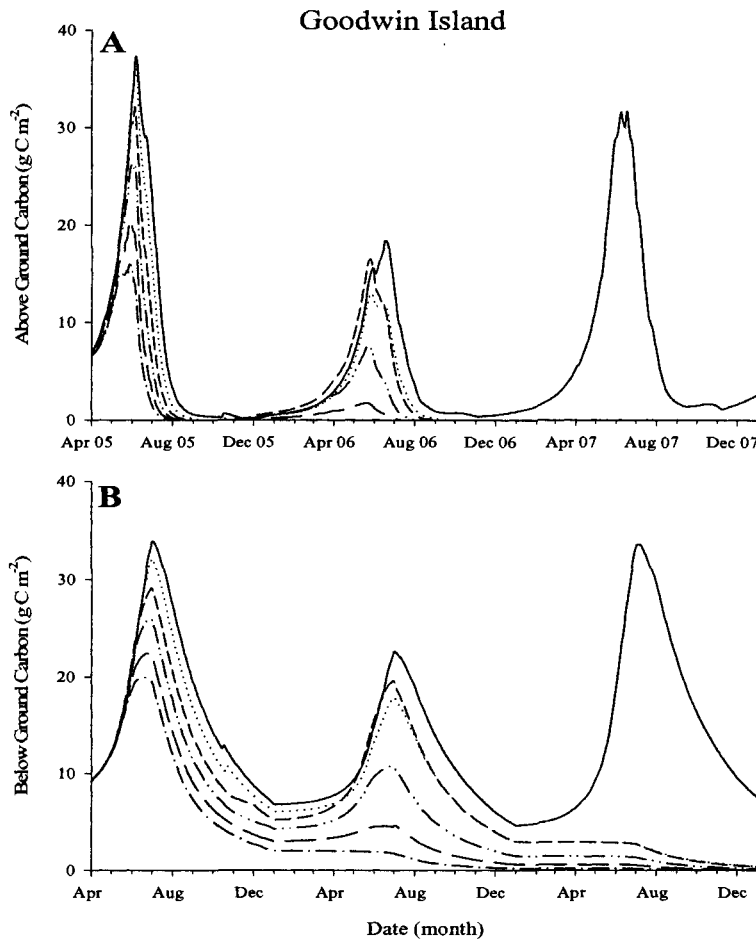


Figure 5-8. Goodwin Island and Gloucester Point model projections with sexual reproduction for 2005 – 2007 under ambient temperatures (solid line), ambient temperatures + 1° C (dotted line), ambient temperatures + 2 °C (short dashed line), ambient temperatures + 3 °C (short dashed and dotted line), ambient temperatures + 4 ° C (long dashed line), and ambient temperature + 5 °C (long dashed and dotted line).

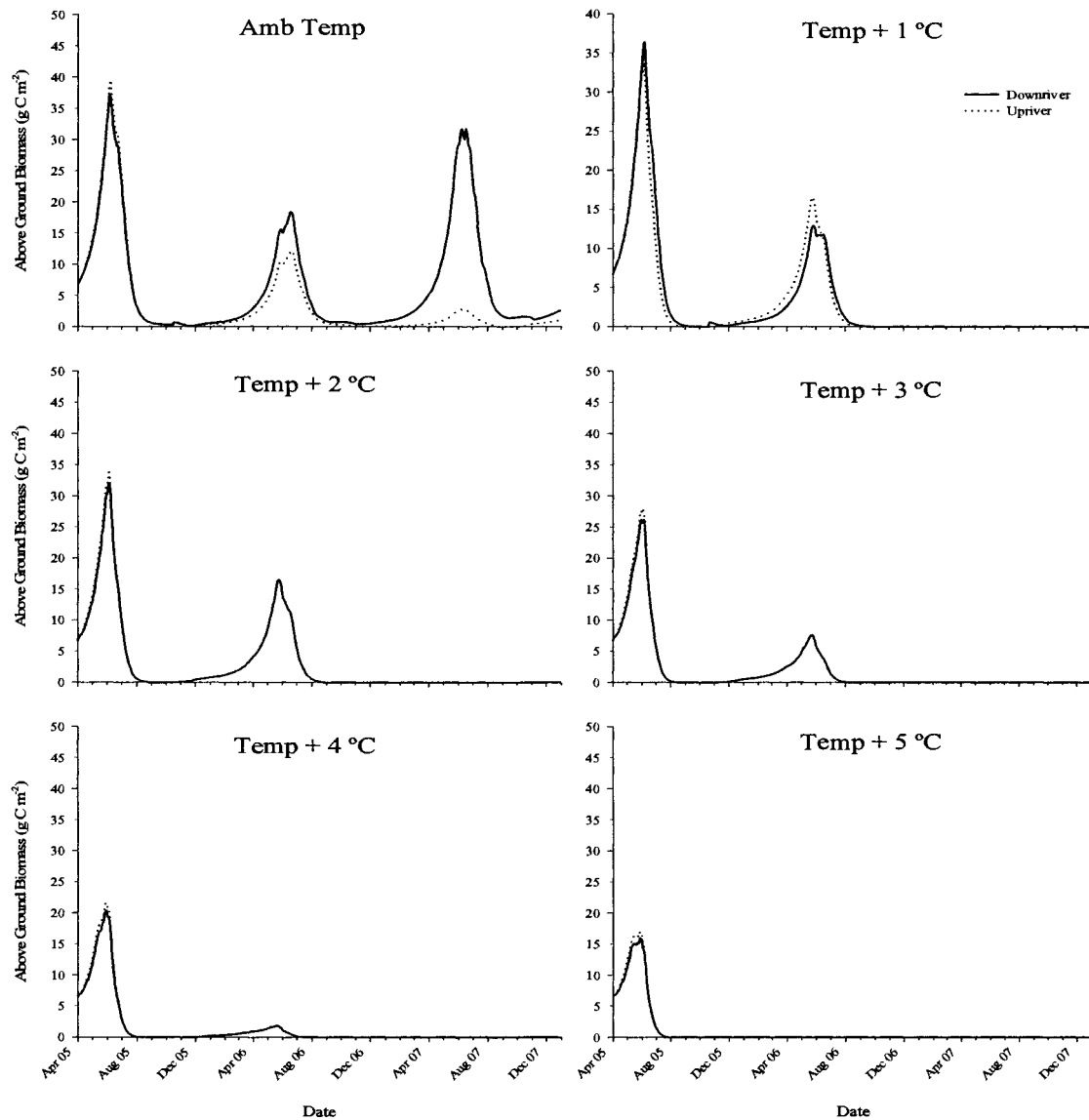


Figure 5-9. Model projections of Goodwin Island above ground biomass with both upriver (GP) water column light attenuation (solid line) and downriver (GI) water column light attenuation (dashed line) conditions under all temperature scenarios.

SYNTHESIS

The main objectives of this research were to (1) quantify and compare responses to various environmental conditions between semi-annual and perennial *Z. marina* populations; (2) quantify the effects of environmental conditions on seed germination and seed-bank viability on the persistence of *Zostera marina* beds using both controlled and *in situ* experiments; (3) to elucidate the role of sexual reproduction in the maintenance and recovery of established *Z. marina* beds; (4) to develop an *Z. marina* production model to more accurately characterize how *Z. marina* beds respond to large scale disturbances and to determine what factors are most important for expanded research relative to seagrass reproduction. The overarching goal of this research was to add to the current understanding of the role of sexual reproduction in seagrass bed maintenance and recovery, and the potential role of seeds and seedlings in seagrass conservation and restoration.

The presence of a semi-annual bed of *Z. marina* at the southern limit of *Z. marina* along the western Atlantic in North Carolina was confirmed. Although semi-annual *Z. marina* beds can be found in habitats which are limiting to perennial *Z. marina* beds, water quality and sediment conditions were not significantly different between semi-annual and perennial *Z. marina* beds in North Carolina suggesting that factors other than these environmental conditions may be related to the development of an semi-annual *Z. marina* bed in this region. In addition, the semi-annual *Z. marina* bed in North Carolina supported a *Z. marina* population with both annual and perennial life histories. *Z. marina* shoots in the semi-annual bed germinated from seeds, a portion of seedlings flowered during their first year of growth, and all shoots completed their life cycle and died within one year of germination like an annual plant; however, not all shoots

flowered and shoots reproduced both sexually and asexually similar to a perennial plant. The development of the mixed life history strategy in North Carolina *Z. marina* bed is not understood and further investigation into genetic variations and phenological response of this population to environmental conditions is required.

Regardless of life history, Virginia and North Carolina seed viability significantly decreased after 6 months in the sediment and both semi-annual and perennial *Z. marina* beds produced transient (seed-bank retains viability < 12 months) seed-banks. The effects of time on seed viability were greater than seed source (semi-annual or perennial) or sediment type ($\geq 90\%$ sand, $\leq 80\%$ sand). Densities of viable seeds within the ambient sediment seed-bank were greater at the semi-annual bed; however, seed-bank viability decreased over time for semi-annual and perennial beds with no viable seeds after 6 months in the sediment. For both life histories ambient seed-bank densities followed *Z. marina* germination cycles and were the lowest following the period of maximum germination (October – November) in December 2007. The significant reduction of seed viability after only 6 months in the sediment suggests that the resiliency provided by seed-banks for both perennial and semi-annual *Z. marina* beds may be limited by seed production on annual scales and by timing of disturbance events.

Seeds from perennial beds in both Virginia and North Carolina had significantly greater maximum germination, shorter time to germination, and greater biomass compared to seeds collected from the semi-annual bed in North Carolina. For seeds from both semi-annual and perennial beds, emergence was greatest in shallow muddy sediments although mean time to germination was not significantly different. Similar to ambient seed-banks, viability of the remaining seeds was not significantly affected by

burial depth or sediment type; however viability for all seed sources was < 10%. While there was no significant regional difference in seedling emergence between seeds from perennial *Z. marina* beds, sediment conditions did significantly affect seedling emergence, highlighting the role of the surrounding sediment rather than the location of the source population on successful sexual reproduction within mid-Atlantic perennial *Z. marina* beds. Reduced seedling emergence of both semi-annual and perennial *Z. marina* seeds at burial depths > 1 cm may represent a possible bottleneck in successful *Z. marina* sexual reproduction. Implications of reduced *Z. marina* seed viability due to burial depth or sediment conditions may affect the resiliency to and recovery from disturbance for both perennial and semi-annual *Z. marina* beds.

Seeds and seedlings played a significant role in the recovery of perennial beds from a sudden and large scale decline. In the summer of 2005 large declines in perennial *Z. marina* populations occurred throughout the lower Chesapeake Bay after prolonged periods of high summer water temperatures. In the spring of 2006 *Z. marina* beds in the York River re-established with seedlings providing 48 – 54 % of the total shoot density. Due to the dominance of seedlings in the spring of 2006, the seed-bank was not replenished. Since seedlings in these regions during their first year of growth are not generally observed to flower and produce seeds, the seed-bank is not immediately replenished and there is limited capacity for bed re-growth. These results demonstrate the importance of seedlings in initial bed recovery following a single disturbance, but highlight potential sensitivity of *Z. marina* beds here to repeated stresses. With this loss of resiliency a repeated disturbance can result in nearly total bed loss.

Ecological models are a useful tool in quantitative analysis of complex ecosystems such as seagrass beds. The loss and recovery processes following a large scale decline in *Zostera marina* in the York River, VA were modeled using an *Z. marina* production model containing a sexual reproduction component. Model projections of *Z. marina* production following the 2005 decline and subsequent recovery period were more accurate relative to *in situ* measurements when sexual reproduction was added compared to the traditional production model with vegetative reproduction only. However, resiliency to increased water temperature provided by sexual reproduction was limited, as a 1 °C increase in temperature resulted in a reduction of biomass to 0 g C m⁻² by year three. Differences in water quality between sites also affected *Z. marina* above and below ground production, seed production, seed viability and seed germination. The results of all model scenarios highlight the need to incorporate sexual reproduction into *Z. marina* ecosystem models, the projected sensitivity of established *Z. marina* beds to consecutive years of stress, and the negative effects of multiple stressors on *Z. marina* recovery.

Sexual reproduction is an important component of both semi-annual and perennial *Zostera marina* populations that should be included in ecological studies and models. Although most perennial *Z. marina* beds rely on asexual reproduction as a primary form of bed maintenance, the ability to reproduce sexually is maintained and, as shown here, may play a large role in the recovery and expansion of these populations. For this reason and due to the use of seeds in restoration of *Z. marina* beds within large systems such as the Chesapeake Bay, research into the dynamics of sexual reproduction within existing beds, a better understanding of seed physiology, and additional research into

environmental effects (including the sediment) on seed germination and viability is required.

VITA

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Jessie was born in Easton, Maryland on July 9, 1981. After graduating from Easton High School in 1999 she went on to earn a B.S. in Biology from Chowan College in 2003. Jessie earned her Masters in Marine Science from the College of William and Mary, School of Marine Science in 2005. Continuing at the College of William and Mary, Jessie graduated with a Doctor of Philosophy in Marine Science from the School of Marine Science in August 2009.